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The Hippo Coactivator TAZ Exacerbates Cisplatin-Induced Acute Renal Injury

Xian Xue[a] Xingwen Zhu[b] Lu Zhou[b] Xiaoli Sun[a] Mengru Gu[b]
Yan Liang[b] Mengzhu Tan[b] Qing Hou[b] Sudan Wang[b] Chunsun Dai[a, b]

[a]Center for Kidney Disease, The 2nd Affiliated Hospital, Nanjing Medical University, Nanjing, China;
[b]Department of Clinical Genetics, The 2nd Affiliated Hospital, Nanjing Medical University, Nanjing, China

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TAZ · PPARδ · Tubular cell ferroptosis · Acute kidney injury

Abstract
Introduction: Transcriptional coactivator with PDZ-binding motif (TAZ), a Hippo signaling pathway effector, maintains the balance of cell proliferation, differentiation, and death. However, the role of TAZ in tubular cell survival and acute kidney injury (AKI) remains largely unknown. ***Methods:*** We used the RNA-seq database, Western blot, and immunohistochemistry to examine TAZ expression in kidneys from cisplatin-induced AKI. We generated tubular-specific TAZ knockout mice to assess the role of TAZ in cisplatin-induced renal toxicity. Immunoprecipitation-mass spectrometry followed standard procedures. ***Results:*** TAZ was activated in tubular cells in kidneys injected with cisplatin. Conditional deletion of TAZ in tubular cells confers ferroptosis resistance and protects kidneys from cisplatin-induced AKI, whereas overexpression of TAZ(S89A) exacerbates cisplatin-induced ferroptosis. Inhibition of ferroptosis with ferrostatin-1 potently preserves renal function and alleviates morphological injury and tubular cell ferroptosis induced by cisplatin. Mechanistically, in a PPARδ-dependent manner, but not TEAD, TAZ reduces the expression of glutathione peroxidase 4 (GPX4), thus exacerbating cisplatin-induced ferroptosis. ***Conclusions:*** Our findings show that cisplatin-induced AKI and tubular cell ferroptosis are mediated by TAZ-PPARδ interaction through regulation of GPX4, highlighting TAZ as a potential therapeutic candidate for AKI.

Introduction

Acute kidney injury (AKI) is a health problem, which is characterized by acute and transient deterioration of kidney function and high mortality, worldwide [1]. Patients with AKI are at high risk of progressing to chronic kidney disease. Pathologically, AKI is characterized by renal tubular injury, peritubular endothelial dysfunction, and inflammatory cell infiltration [2]. The precipitating factors of clinical AKI include ischemia, sepsis, and renal toxic reagents [3, 4]. Cisplatin is a commonly used chemotherapeutic drug, which is a nephrotoxic agent that induces AKI in up to 31.5% of the patients [5]. Renal proximal tubular cells are responsible for the uptake and excretion of cisplatin. Cisplatin accumulation results in cell death and inflammation [6, 7]. Therefore, deciphering the underlying mechanisms of tubular cell survival is essential for preventing AKI and treating patients with AKI.

Xian Xue, Xingwen Zhu, and Lu Zhou contributed equally to this work.

karger@karger.com
www.karger.com/kdd

Correspondence to:
Chunsun Dai, daichunsun@njmu.edu.cn

The Hippo signaling pathway is an evolutionarily conserved kinase cascade that controls the balance of cell differentiation, proliferation, and death, and, therefore, determines the organ size [8]. The core components and downstream effectors of the Hippo pathway in mammals include Mst1/2, Sav1, Lats1/2, Yes-associated protein (YAP), and its paralogue PDZ-binding motif (TAZ) [9]. Phosphorylation and inhibition of the transcriptional coactivators, YAP and TAZ, limit organ size, whereas they play an essential role in the regulation of proliferation and apoptosis after translocation into the nucleus [10]. TAZ, also called WWTR1, serves as an important transcriptional coactivator and interacts with several transcription factors, including TEAD, RUNX1/2, and PPARγ [11–14]. Laboratory and clinical research studies have demonstrated the significant role of TAZ in human-associated diseases such as cancer and kidney disease [15, 16]. However, the possible role of TAZ in cisplatin-induced AKI remains unknown.

Ferroptosis, a recently identified form of iron-dependent regulated cell death, is characterized by the accumulation of lipid reactive oxygen species (ROS) [17]. Glutathione deprivation and inhibition of glutathione peroxidase 4 (GPX4) ultimately result in overwhelming lipid peroxidation that can trigger ferroptosis [18, 19]. Induction of GPX4 deletion in mice results in lipid oxidation-induced acute renal failure and associated death [20, 21]. Ferroptosis dysfunction is strongly related to pathological cell death, including ischemia-reperfusion injury (IRI) [22]. In addition, it has been proven that ferroptosis contributes to damage in nephrotoxic FA-induced and cisplatin-induced AKI, as ferrostatin-1 (Fer-1) improves renal function and decreases intracellular lipid peroxidation, tubular cell injury, and death [23–25]. In epithelial tumors, TAZ is a novel driver of ferroptosis [26, 27]. Thus, the idea that multiple inhibitors of ferroptosis hold great potential for AKI therapy seems more probable. As such, there is growing interest in the identification of the molecular mechanisms involved in the regulation of ferroptosis in AKI. This study aimed to assess the role of TAZ in cisplatin-induced renal toxicity by generating tubular-specific TAZ knockout mice.

Methods

Animals

$TAZ^{fl/fl}$ mice were kindly provided by Dr. Randy L. Johnson from MD Anderson Cancer Center. $TEAD1^{fl/fl}$ mice were ordered from Cyagen (Suzhou, China). Mice with genotypes Cre+/–, $TAZ^{fl/fl}$ (Tubule-TAZ–/–) and Cre+/–, $TEAD1^{fl/fl}$ (Tubule-TEAD1–/–) were generated by cross-breeding Ksp1.3/Cre transgenic mice (cat: 012237, Jackson Laboratory) with $TAZ^{fl/fl}$ and $TEAD1^{fl/fl}$, respectively (all were on C57BL/6 background). The same gender mice genotyping Cre–/–, $TAZ^{fl/fl}$ (Tubule-TAZ+/+) and Cre–/–, $TEAD1^{fl/fl}$ (Tubule-TEAD1+/+) from the same litters were considered control littermates. Genotyping was performed by PCR using DNA extracted from mouse tails. To induce the AKI, mice aged between 6 and 8 weeks were injected with a single dose of cisplatin (20 mg/kg) (cat: P4394, Sigma-Aldrich, St. Louis, MO, USA) intraperitoneally. Mice were killed on day 3 after cisplatin administration. Blood and kidney samples were harvested for further analysis.

Male CD1 mice aged 6–8 weeks and weighing ~18–20 g were acquired from the Specific Pathogen-Free (SPF) animals at the Laboratory Animal Center of Nanjing Medical University. To induce the AKI model in vivo, CD1 mice were injected intraperitoneally with a single dose of cisplatin (20 mg/kg) to induce AKI. Mice were killed on days 1, 2, and 3 after cisplatin administration. Mice were injected intraperitoneally with Fer-1 (5 mg/kg) (catalog no. SML0583, Sigma-Aldrich) about 45 min prior to cisplatin injection. All animals were maintained in SPF conditions at the Laboratory Animal Center of Nanjing Medical University according to the guidelines of the Institutional Animal Care and Use Committee from Nanjing Medical University.

Statistical Analysis

All data examined are presented as mean ± SEM. Statistical analyses of the data were performed using the SigmaStat software (Jandel Scientific Software, San Rafael, CA, USA). Comparison between groups was made using a one-way analysis of variance, followed by the Student-Newman-Keuls test. Paired or unpaired *t* test was used to compare the two groups. $p < 0.05$ was considered statistically significant.

Results

Induction of TAZ in Mouse Kidneys with Cisplatin-Induced AKI

To identify the molecular mechanisms underlying cisplatin-induced AKI, we first assessed the global transcriptomic changes in the mouse kidney following cisplatin treatment using the high-throughput sequencing (RNA-seq) dataset, GSE106993. Global transcriptomic analysis revealed that the Hippo

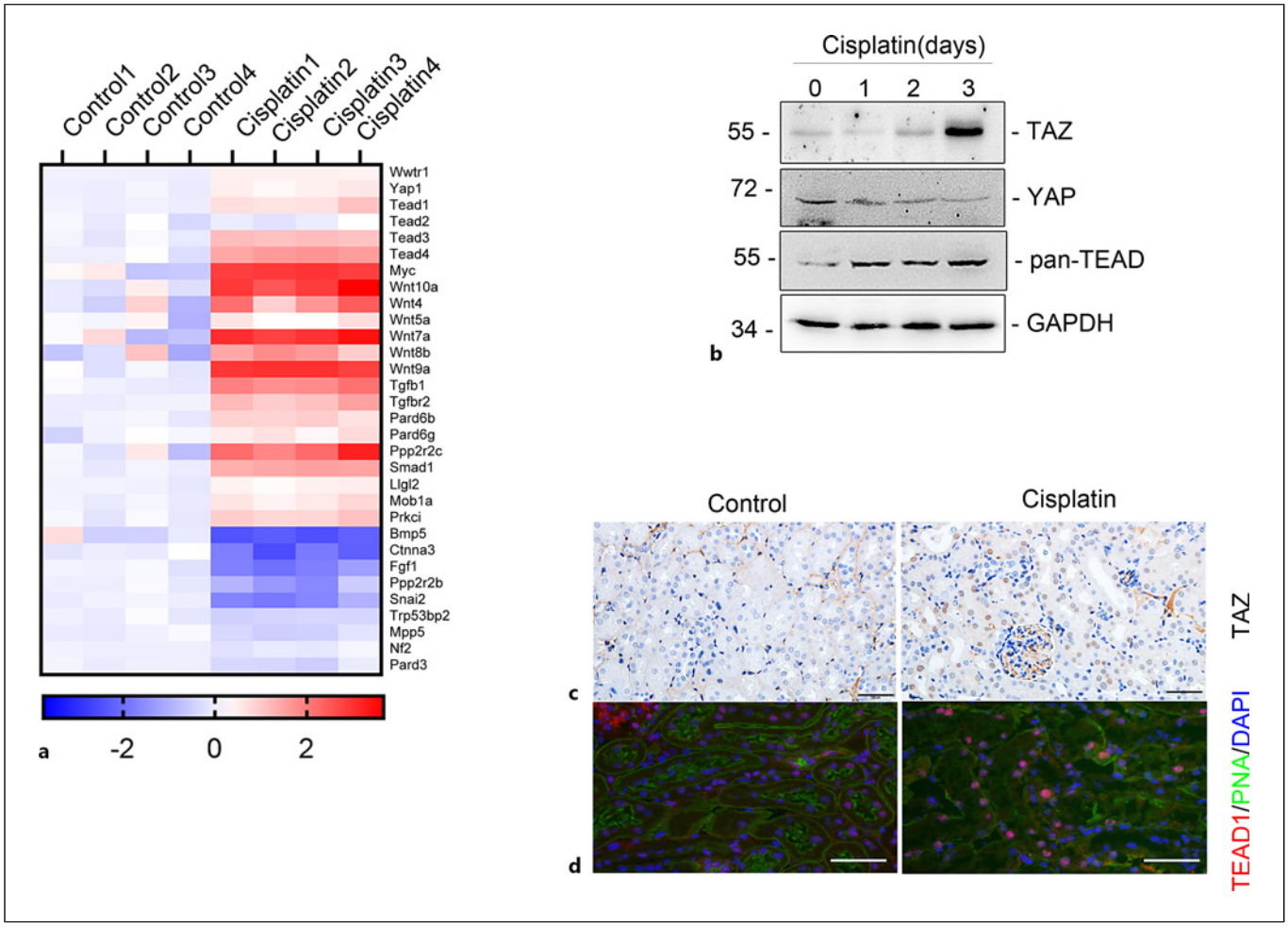


Fig. 1. Induction of TAZ in mouse kidneys with cisplatin-induced AKI. **a** Heatmap showing the gene expression of Hippo signaling pathway in a mouse model with cisplatin treatment obtained from GSE106993. **b** Western blotting analyses showing the expression of TAZ, YAP, and pan-TAED in kidneys from mice with cisplatin injection at different times as indicated. Immunohistochemical staining of TAZ (**c**) and immunofluorescence co-staining of anti-TEAD1 with FITC-PNA (**d**) in kidney tubule from mice on day 3 after cisplatin injection. Scale bar, 20 μm.

signaling pathway had been dynamically suppressed, with upregulated mRNA levels of TAZ, YAP, and TEAD, in mice that were treated with cisplatin (shown in Fig. 1a). As expected, obviously upregulation of TAZ protein expression was observed after day 3 of cisplatin treatment. Similarly, pan-TEAD protein expression increased within the time frame of the experiment. However, the expression of YAP was reduced after cisplatin injection (shown in Fig. 1b). Immuno-staining showed that the induction of TAZ and TEAD1 were mainly localized in the tubule epithelial cell nuclei (shown in Fig. 1c, d). Together, these data indicate that TAZ is activated in cisplatin-induced renal injury.

Specific Deletion of TAZ in Tubular Cells Protects against Cisplatin-Induced AKI

To investigate the specific role of TAZ in tubular cells in vivo, we created a mouse model with TAZ deficiency in tubular cells using the Cre-LoxP system (shown in online suppl. Fig. S1a; for all online suppl. material, see https://doi.org/10.1159/000540973). By cross-breeding of TAZ-floxed mice and Ksp-Cre transgenic mice, we obtained knockout mice with genotype Ksp-Cre+/−, TAZ f/f (Tubule-TAZ−/−, shown in online suppl. Fig. S1b, lane 1). Littermates with genotype Ksp-Cre−/−, TAZ fl/fl were used as controls (Tubule-TAZ+/+, shown in online suppl. Fig. S1b, lane 2). The Ksp-Cre recombinase is mainly expressed in collecting ducts, Heinz's loops, and distal

convoluted tubules, partially expressed in the proximal tubule [28, 29]. Immunofluorescent staining showed that TAZ expression in proximal tubular cell (FITC-PHA-E) or distal tubular cell (FITC-PNA) was markedly reduced in mice with tubular cell-specific TAZ deletion (shown in online suppl. Fig. S1c, d). The kidney/body weight ratio and blood urea nitrogen (BUN) level were comparable between Tubule-TAZ−/− and Tubule-TAZ+/+ mice at 2 months after birth (shown in online suppl. Fig. S1e, f).

We then investigated the functional significance of tubule-specific TAZ with both Tubule-TAZ−/− and Tubule-TAZ+/+ mice that were injected with cisplatin. No significant differences were found in kidney histology in Tubule-TAZ+/+ and Tubule-TAZ−/− mice (shown in Fig. 2b). On day 3 after cisplatin injection, an obvious increase in BUN level and morphological injury were observed in Tubule-TAZ+/+ mice, whereas kidney dysfunction and morphological abnormalities were attenuated in Tubule-TAZ−/− mice (shown in Fig. 2a–c). Analysis of regulated cell death, as determined by TUNEL assay, revealed a higher number of TUNEL-positive cells in the kidneys of cisplatin-treated Tubule-TAZ+/+ mice compared with Tubule-TAZ−/− mice (shown in Fig. 2d, e). Together, these results demonstrate that ablation of endogenous TAZ in tubular cells protects kidneys from cisplatin-induced AKI.

Ferroptosis Plays an Essential Role in Mediating Cisplatin-Induced AKI

The global transcriptomic change analysis between the kidneys treated with cisplatin and the vehicle revealed that among the various differentially regulated signaling pathways, the ferroptosis pathway was found to be significantly activated in cisplatin-treated mice (shown in Fig. 3a). Thus, we sought to validate the role of ferroptosis in the kidney with cisplatin-induced AKI and used an immunohistochemistry analysis to examine ferroptosis biomarkers in cisplatin-treated kidneys. The expression of GPX4 was maximally detected in the tubular cells of the control kidneys, while GPX4 was significantly downregulated in the kidneys after cisplatin treatment (shown in Fig. 3b, f). The protein abundance of GPX4 was decreased in mouse renal tissue lysates on day 1 and the maximum reduction was seen on day 3 after cisplatin injection (shown in Fig. 3e). Concomitantly, immunohistochemistry analysis of the kidneys by 4-hydroxynonenal (4-HNE) staining confirmed an increase of lipid peroxidation in cisplatin-treated renal failure (shown in Fig. 3c, g). Using transmission electron microscopy, we observed that renal tubule epithelial cells, treated with cisplatin, exhibited smaller mitochondria with condensed membrane density, which are the characteristic morphologic features of cells undergoing ferroptosis (shown in Fig. 3d).

To further validate the role of ferroptosis in cisplatin-induced AKI, we pretreated mice with Fer-1, a specific inhibitor of ferroptosis, for about 45 min prior to cisplatin treatment. On day 3 after cisplatin injection, a rapid increase in blood urea level, enlargement of tubular lumen, and tubular cell death and detachment were observed. Notably, BUN levels and morphological injury were largely regressed in mice pretreated with Fer-1 (shown in Fig. 3h–j). Therefore, these results suggest that ferroptosis plays an essential role in cisplatin-induced AKI.

Pharmacologically Inhibition of Ferroptosis Attenuates Cisplatin-Induced Tubular Cell Death

In line with the above results, the induction of TAZ was significantly increased and the expression of GPX4 was remarkably suppressed in a time-dependent manner in cisplatin-treated NRK-52E cells (shown in Fig. 4a). Then, we assessed whether Fer-1 could prevent the cell death induced by cisplatin. As expected, Fer-1 fully prevented NRK-52E cell death in the presence of cisplatin, as determined by propidium iodide (PI) staining (shown in Fig. 4b, c). Indeed, overexpression of GPX4 significantly attenuated cisplatin-induced morphological changes and cell death (shown in Fig. 4d–f). These results confirm that ferroptosis plays a key role in cisplatin-induced cell death and GPX4 plays a key regulator of ferroptosis in response to cisplatin.

TAZ Gene Disruption Alleviates Tubular Cell Ferroptosis in Cisplatin-Induced AKI

Previously published observations suggested that the Hippo-YAP/TAZ pathway is a novel determinant of ferroptosis in epithelial tumors [26, 30]. Thus, we sought to investigate the role of TAZ in the regulation of cisplatin-induced ferroptosis. Ultrastructural analysis revealed shrunken mitochondria in tubule epithelial cells of cisplatin-treated Tubule-TAZ+/+ mice, whereas mitochondrial defects were ameliorated in the tubular cells in Tubule-TAZ−/− mice (shown in Fig. 5a). We then used dichloro-dihydro-fluorescein diacetate probe fluorescence to detect the mitochondrial ROS level in the kidneys from both genotypes of mice. Notably, increased levels of mitochondrial ROS were observed in the kidneys of cisplatin-treated Tubule-TAZ+/+ mice, and the levels were attenuated in Tubule-TAZ−/− mice (shown in Fig. 5b, e).

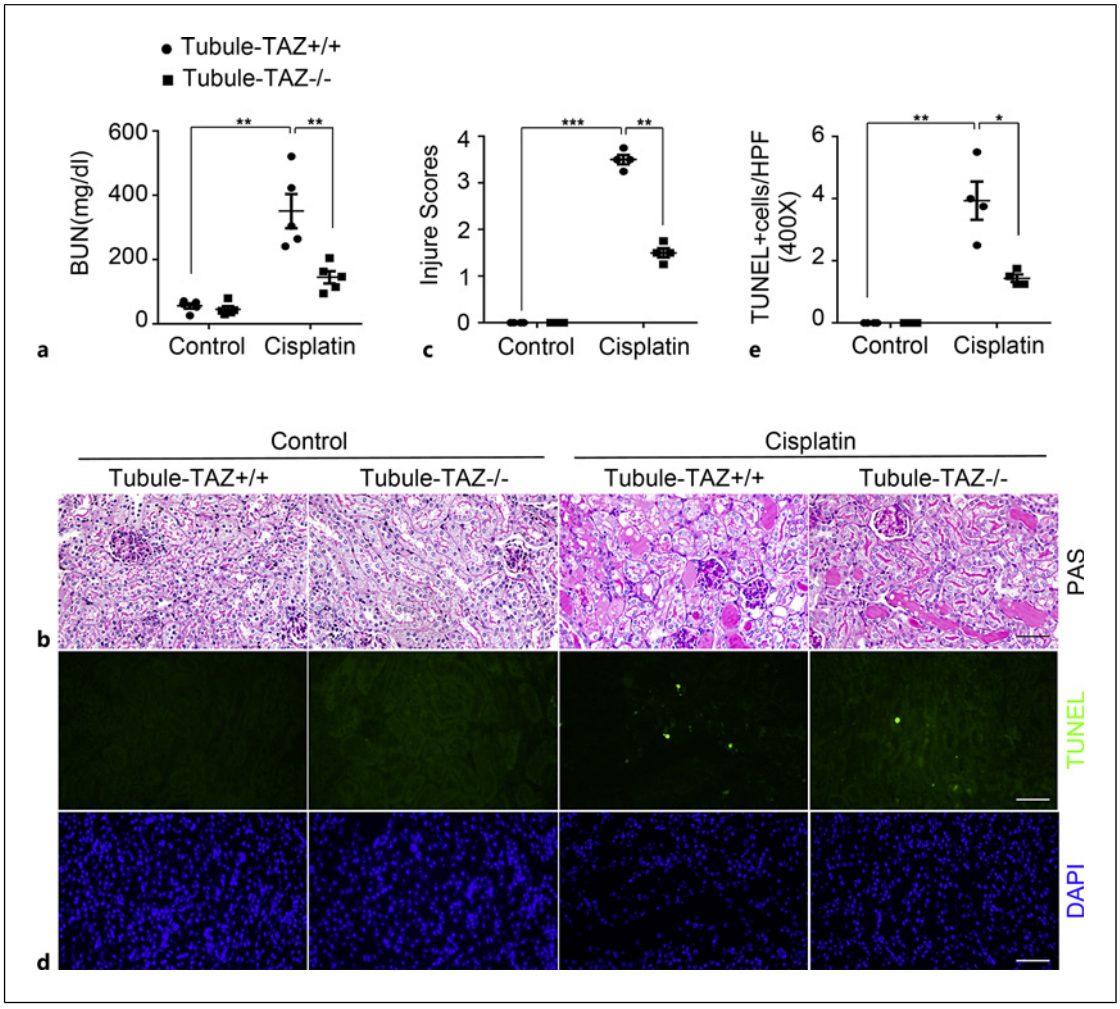


Fig. 2. Specific deletion of TAZ in tubular cells protects against cisplatin-induced AKI. **a** The graph showing blood urea nitrogen (BUN) level of Tubule-TAZ–/– and Tubule-TAZ+/+ mice on day 3 after cisplatin injection. **b** Kidney histology as shown by periodic acid-Schiff (PAS) staining. Scale bar, 20 μm. **c** Injury scores. Each dot represents the average of five HPFs from each mouse. **d** Representative micrographs showing TUNEL staining. Scale bar, 20 μm. **e** Quantitative analysis of TUNEL-staining positive cells among groups as indicated. Data are presented as means ± SEM (n = 4–5 for each group). *p < 0.05, **p < 0.01, ***p < 0.001.

Histochemical analysis of the kidneys by GPX4 and 4-HNE staining revealed greater resistance to lipid peroxidation in the cisplatin-treated Tubule-TAZ–/– mice compared to that in Tubule-TAZ+/+ mice, while basal levels of both proteins were not significantly affected in the absence of cisplatin (shown in Fig. 5c, d and f). Furthermore, GPX4 expression was significantly decreased in cisplatin-treated Tubule-TAZ+/+ mice, while it was remarkably upregulated in Tubule-TAZ–/– mice (shown in Fig. 5g, h). Thus, these data suggest that selective tubular-TAZ deletion attenuates tubular injury induced by cisplatin through antagonizing ferroptosis.

TAZ Deficiency Mitigates Cisplatin-Induced Tubular Cell Death through Upregulation of GPX4

To further investigate the effect of TAZ on tubular cell ferroptosis, we evaluated cisplatin-induced ferroptosis in primary cultured tubular cells (PCTCs) isolated from TAZ f/f mice, which were infected with adenovirus carrying Cre recombinase (Ad-Cre) to induce TAZ gene ablation and they were named Tubule-TAZ–/– cells. The tubular cells infected with adenovirus carrying GFP (Ad-GFP) were considered control cells (Tubule-TAZ+/+). As shown in Figure 6a, we confirmed, by Western blot analysis, that TAZ protein abundance was dramatically reduced in

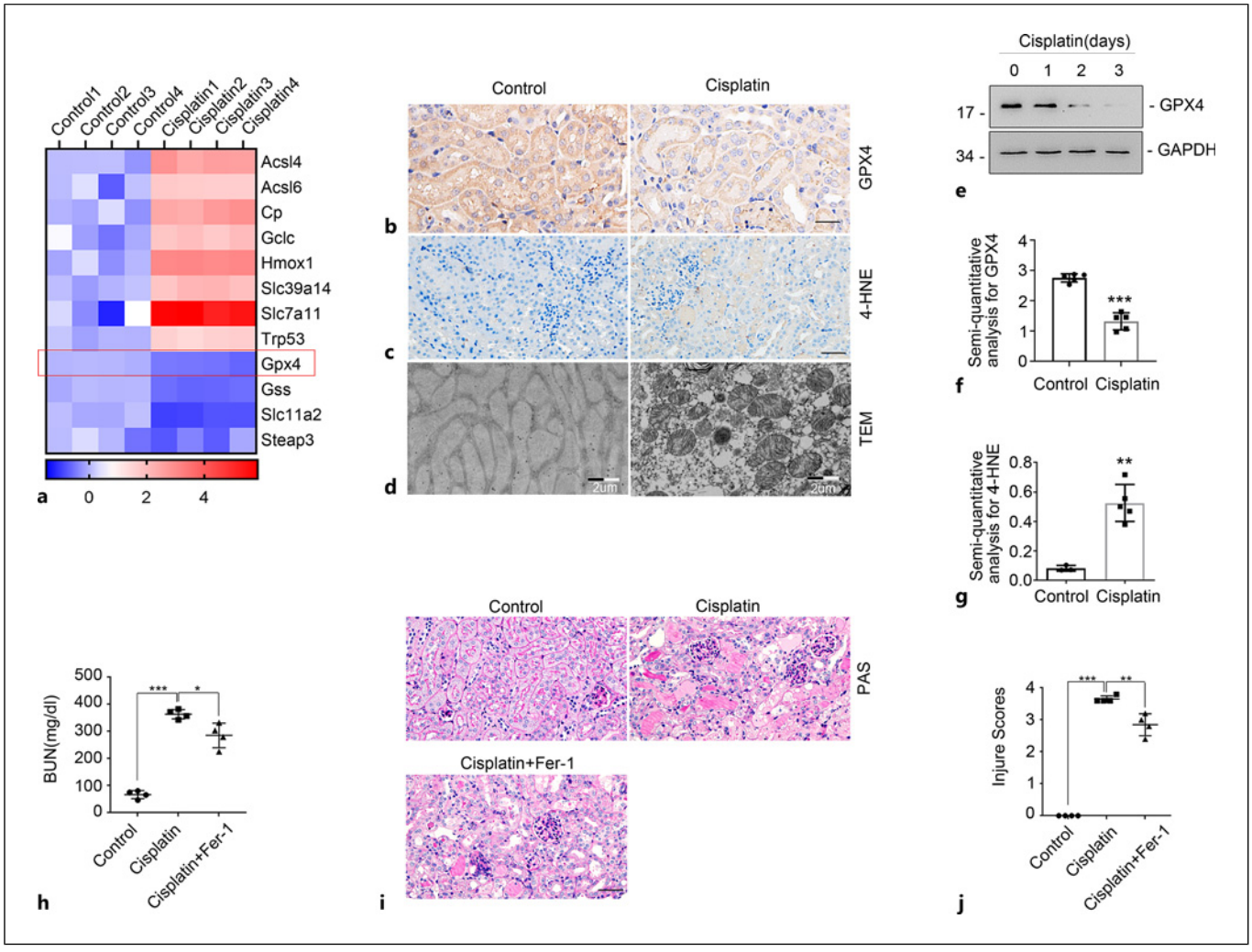


Fig. 3. Ferroptosis plays an essential role in mediating cisplatin-induced AKI. **a** Heatmap showing the gene expression of the ferroptosis pathway in a mouse model with cisplatin treatment obtained from GSE106993. Representative micrographs for GPX4 (**b**) and 4-HNE (**c**) staining in kidney tissues from mice on day 3 after cisplatin injection (scale bar, 20 μm); representative transmission electron micrographs (**d**) of tubular cells as indicated (scale bar, 2 μm). **e** Western blot assay showing the expression of GPX4 in kidneys from mice after cisplatin injection. Graphic presentation showing the semi-quantitative analyses for GPX4 (**f**) and 4-HNE (**g**). **h** The graph showing the BUN level of each group on day 3 after cisplatin injection. **i** Kidney histology as shown by periodic acid-Schiff (PAS) staining. Scale bar, 20 μm. **j** Injury scores. Each dot represents the average of five HPFs from each mouse. Data are presented as means ± SEM (n = 4–5 for each group). *p < 0.05, **p < 0.01, ***p < 0.001.

Tubule-TAZ−/− cells compared to that in Tubule-TAZ+/+ cells. After adenovirus infection, both Tubule-TAZ+/+ and Tubule-TAZ−/− PCTCs showed a cobblestone epithelial morphology (shown in Fig. 6b). PI staining revealed that cisplatin treatment led to massive cell death, which was alleviated by TAZ deficiency (shown in Fig. 6b, c). Moreover, loss of function of TAZ resulted in decreased lipid peroxidation, as determined by 4-HNE, in the presence of cisplatin treatment (shown in Fig. 6d).

We next elucidated how the co-transcription factor, TAZ, regulates ferroptosis. Immunoblotting assay showed that TAZ deletion obviously upregulated GPX4 expression, which was decreased by cisplatin treatment (shown in Fig. 6d). Furthermore, the mRNA abundance of GPX4 was approximately 1.5-fold higher with ablation of TAZ compared to that in the control group (shown in Fig. 6e). To further evaluate the role of GPX4 in TAZ-regulated ferroptosis, PCTCs were transfected with GPX4 small interfering RNAs (siRNAs) to knockdown the

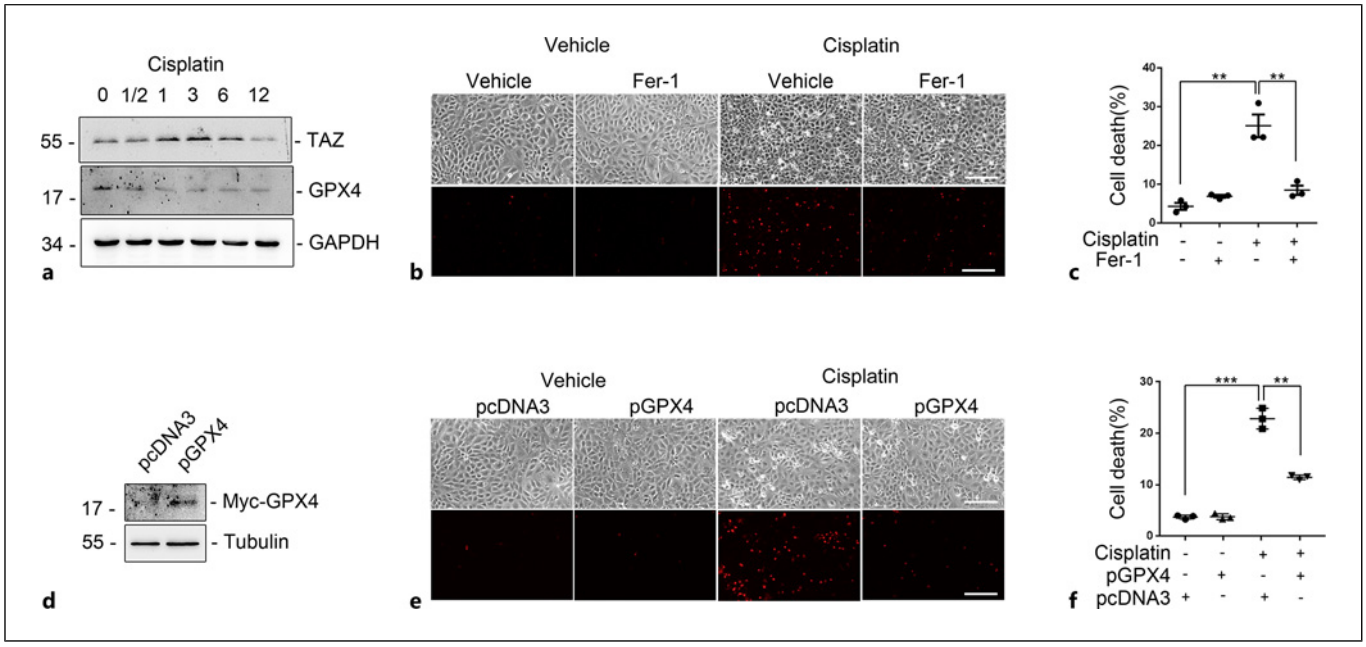


Fig. 4. Pharmacologically inhibition of ferroptosis attenuates cisplatin-induced tubular cell death. **a** Western blot assay showing the expression of GPX4 and TAZ in NRF-52E cells treated with cisplatin for different times as indicated. **b** Phase-contrast images and PI staining of NRF-52E cells treated with cisplatin with or without ferrostatin-1 (Fer-1) for 12 h. Independent experiments were repeated three times and representative data are shown. Scale bar, 20 μm. **c** Quantitative analysis of dead cells. Data are presented as the percentage of PI-staining positive cells. **d** Western blotting analyses showing the abundance of Myc-GPX4 in NRF-52E cells. **e** Phase-contrast images and PI staining showing the NRF-52E cells transfected with pGPX4 plasmid treated with cisplatin for 12 h. Scale bar, 20 μm. **f** Quantitative analysis of dead cells. Data are presented as the percentage of PI-staining positive cells. Data are presented as means ± SEM (n = 3 for each group). $^{**}p < 0.01$, $^{***}p < 0.001$.

expression of GPX4 (shown in Fig. 6f). Indeed, loss of GPX4 further aggravated the number of cisplatin-triggered cell death in the Tubular-TAZ+/+ group, while cell death was alleviated by ablation of TAZ, suggesting that GPX4 is involved in TAZ-regulated ferroptosis (shown in Fig. 6g, h).

Forced Expression of TAZ Exacerbates Cisplatin-Induced Tubular Cell Ferroptosis through Downregulation of GPX4

To further elucidate the mechanism of TAZ in ferroptosis in vitro, we transfected NRK-52E cells with a plasmid carrying a constitutively active form of TAZ (TAZ-S89A). Forced expression of TAZ was confirmed by immunoblotting procedures (shown in Fig. 7a). As expected, NRK-52E cells with expression of TAZ(S89A) showed decreased mRNA and protein abundance of GPX4 compared to the control group (shown in Fig. 7b, c). Interestingly, the effect of cisplatin-triggered GPX4 expression inhibition was dramatically enhanced in cells with overexpression of TAZ, compared to that in control cells (shown in Fig. 7c). Notably, expression of TAZ(S89A) exacerbated the severity of cell death caused by cisplatin. Moreover, overexpression of GPX4 significantly mitigated the cisplatin-induced cell death compared to overexpression of TAZ (shown in Fig. 7d, e). Together, our data further suggest that TAZ regulates ferroptosis induced by cisplatin through GPX4.

TEAD Is Not Required for TAZ to Regulate Tubular Cell Ferroptosis Induced by Cisplatin

Since TEADs have been recognized as the major transcription factors mediating TAZ-regulated downstream target gene expression, we asked whether TAZ regulates tubular cell ferroptosis through TEADs. All TEAD family members but TEAD2 were expressed at a high level in NRK-52E cells. Surprisingly, downregulation of TEAD1, TEAD3, and TEAD4 with siRNAs transfection in NRK-52E cells promoted cisplatin-induced tubular cell death (shown in Fig. 8a–c online suppl. Fig. S2a–d).

We then generated a mouse model with TEAD1 deficiency in tubular cells using the Cre-LoxP system

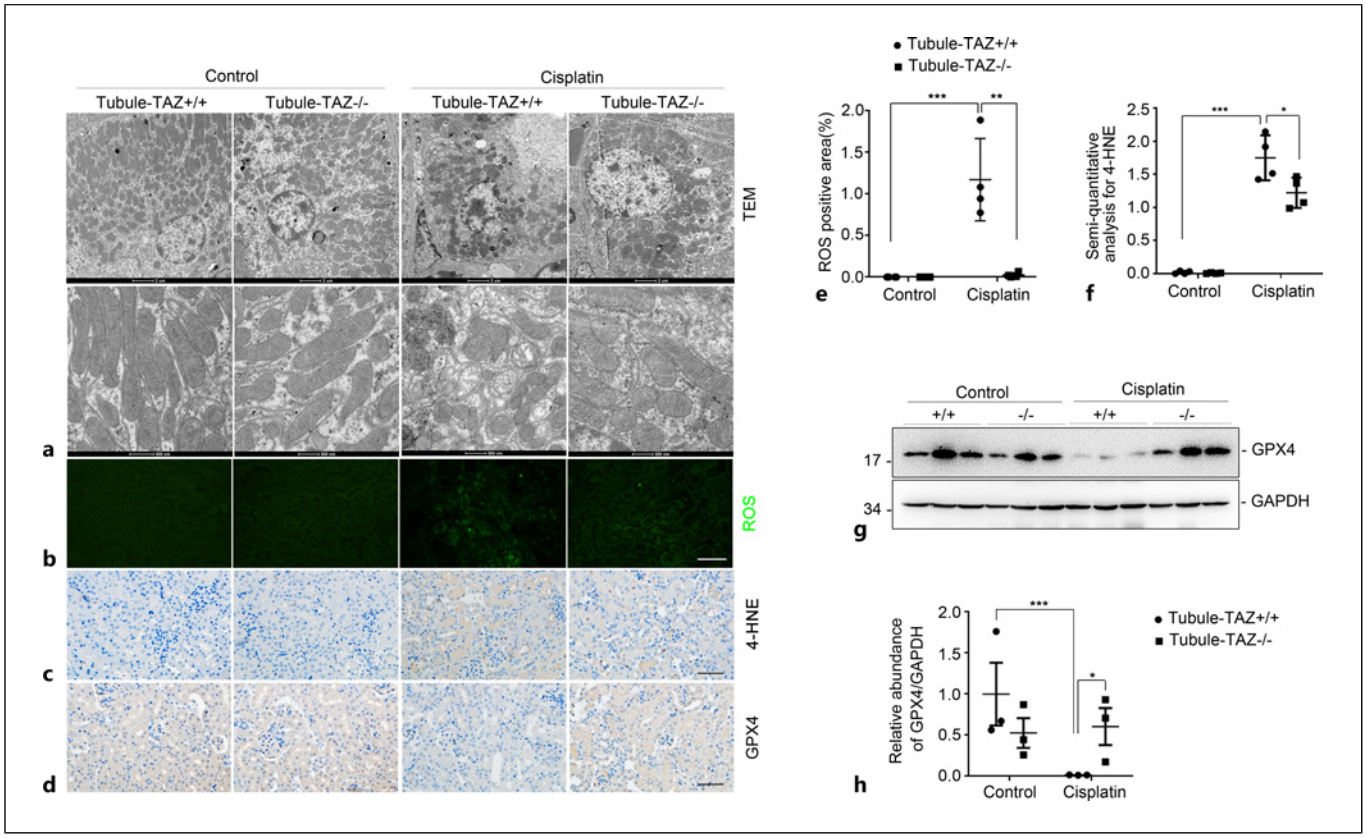


Fig. 5. TAZ gene disruption alleviates tubular cell ferroptosis in cisplatin-induced AKI. **a** Representative transmission electron micrographs of tubular cells from Tubule-TAZ+/+ and Tubule-TAZ–/– mice treated with cisplatin. Scale bar, 2 μm, 500 nm. **b** Representative micrographs showing DCFH-DA staining for ROS in kidney tissues. Scale bar, 20 μm. **c**, **d** Representative immunohistochemistry staining images showing the expression of GPX4 and 4-HNE in kidney tissues from mice on day 3 after cisplatin injection. **e** Quantitative analysis of DCFH-DA staining positive area among groups as indicated. Data are presented as the percentage of the staining positive area. Each dot represents the average of five HPFs from each mouse. **f** Semi-quantitative analyses for 4-HNE. Western blot assay (**g**) and quantitative analyses (**h**) showing the expression of GPX4 in kidneys from Tubule-TAZ+/+ and Tubule-TAZ–/– mice after cisplatin injection. Data are presented as means ± SEM (n = 3–4 for each group). *p < 0.05, **p < 0.01, ***p < 0.001.

(shown in online suppl. Fig. S3a, b). Immunofluorescent staining confirmed the ablation of TEAD1 in proximal tubular cells (FITC-PHA-E) or distal tubular cells (FITC-PNA) (shown in online suppl. Fig. S3c, d). No kidney injury was observed in Tubule-TEAD1–/– mice at 2 months after birth (shown in online suppl. Fig. S3e, f). We then injected the mice with cisplatin to induce AKI. On day 3 after cisplatin injection, BUN level, morphological injury and TUNEL-positive cells were obviously higher in Tubule-TEAD1–/– mice compared with Tubule-TEAD1+/+ mice (shown in Fig. 8d–g). Together, these results demonstrate that ablation of TEAD1 in tubular cells aggravates cisplatin-induced AKI, which suggests that TEAD1 does not mediate TAZ-regulated tubular cell ferroptosis induced by cisplatin.

TAZ and PPARδ Collaboratively Regulate GPX4 Expression and Ferroptosis in Tubular Cells

Our study revealed that TAZ is a novel and direct regulator of GPX4 expression. Furthermore, we sought to identify how TAZ regulated *GPX4* gene expression. To identify candidate transcription factors in an unbiased fashion, immunoprecipitation-mass spectrometry (IP-MS) was performed in NRK-52E cells with stable transfection of TAZ(S89A). As expected, nine proteins of transcriptional regulators were identified in TAZ-transfected cells using TAZ antibody pulldown but not in normal IgG pulldown (data not shown). Interestingly, the nuclear receptor PPARδ was among these nine candidate proteins (shown in Fig. 9a).

One plausible hypothesis is that TAZ binds to PPARδ and this complex suppresses transcription of GPX4. To

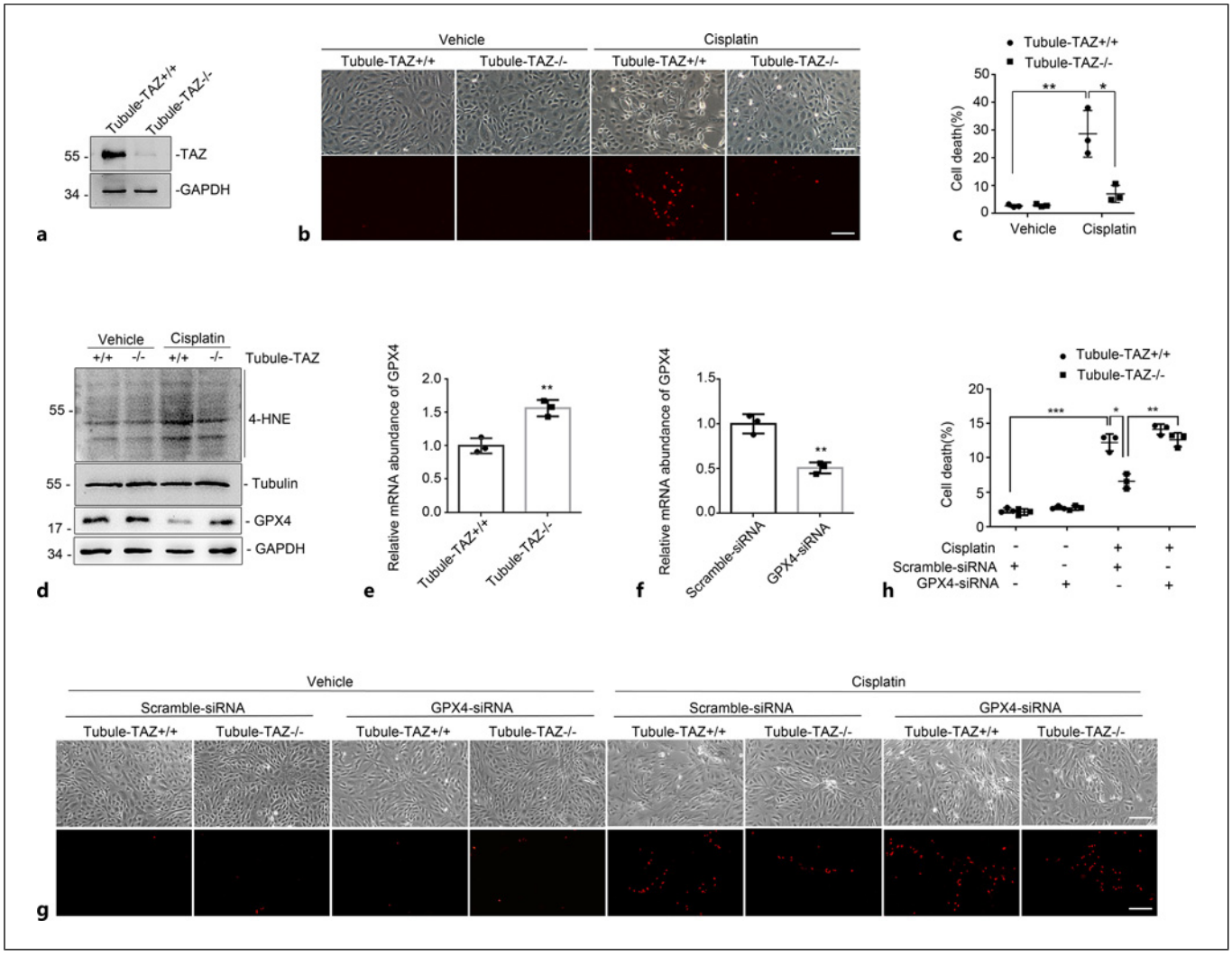


Fig. 6. TAZ deficiency mitigates cisplatin-induced tubular cell death through upregulation of GPX4. **a** Western blot assay showing the reduction of TAZ expression in primary cultured tubular cells (PCTCs) with TAZ ablation. The primary cultured tubular cells from TAZ fl/fl mice were infected with adenovirus carrying GFP or Cre recombinase gene for 48 h, respectively. **b** Phase-contrast micrographs and PI staining of PCTCs incubated with cisplatin for 12 h. Scale bar, 20 μm. **c** Quantitative analysis of dead cells. Data are presented as the percentage of PI-staining positive cells. **d** The protein expression of 4-HNE and GPX4 were assayed as indicated. **e**, **f** Real-time qRT-PCR analysis showing the mRNA abundance of GPX4 as indicated. **g** Representative micrographs showing the phase contrast and PI staining as indicated. Scale bar, 20 μm. **h** Quantitative analyses of dead cells. Data are presented as means ± SEM (n = 3 for each group). *p < 0.05, **p < 0.01, ***p < 0.001.

test this hypothesis, we performed immunoprecipitation experiments in NRK-52E cells. As expected, PPARδ protein precipitated with TAZ (shown in Fig. 9b, c). Furthermore, in reverse experiments, TAZ protein was precipitated by the PPARδ antibody in cell lysates (shown in Fig. 9b). Interestingly, overexpression of TAZ led to a substantial increase in PPARδ protein expression in NRK-52E cells and PCTCs (shown in Fig. 9c and online suppl. Fig. S4a). Next, we facilitated siRNA-mediated depletion of PPARδ. As expected, mRNA levels of PPARδ were substantially diminished, while there was no change in the mRNA levels of other subtypes of PPAR, verifying the specificity of PPARδ siRNA (shown in Fig. 9d). Furthermore, Western blot analysis also confirmed that PPARδ protein abundance was robustly reduced in NRK-52E cells and PCTCs (shown in Fig. 9e and online suppl. fig. S4b). Indeed, siRNA-mediated loss of PPARδ resulted in upregulated GPX4 protein abundance in over-expressed TAZ in both types of cells (shown in Fig. 9f and online suppl. Fig. S4c). Moreover, the loss of

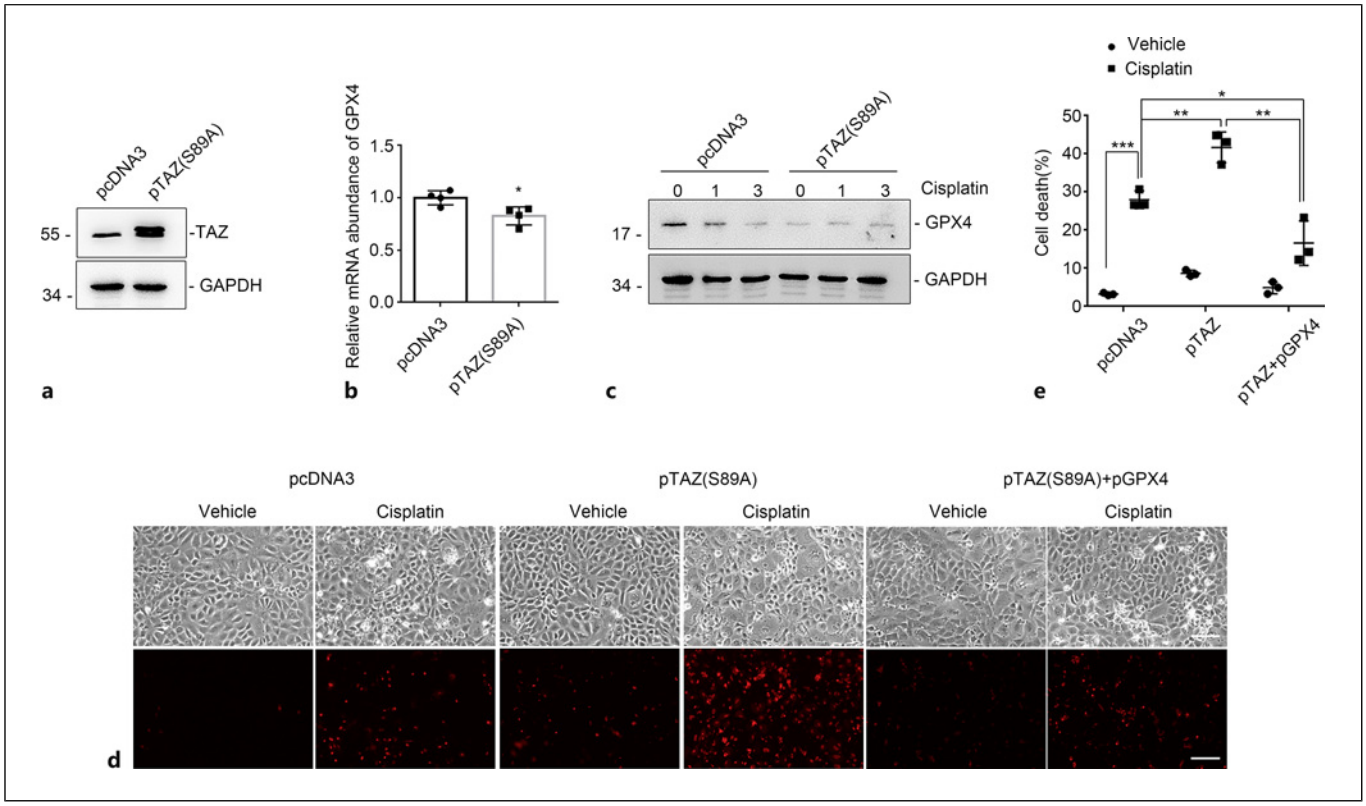


Fig. 7. Forced expression of TAZ exacerbates cisplatin-induced tubular cell ferroptosis through downregulation of GPX4. **a** Western blotting analysis of the expression of exogenous TAZ(S89A) in NRF-52E cells after TAZ(S89A) plasmid transfection. **b** The mRNA abundance of GPX4 in NRF-52E cells after TAZ(S89A) plasmid transfection. The relative gene expression is normalized to GAPDH. **c** Western blot assays showing the abundance of GPX4 in cells. NRF-52E cells were transiently transfected with pTAZ(S89A), followed 24 h later treated with cisplatin for 1 and 3 h. **d** Phase-contrast images and PI staining were assayed in indicated cells treated with cisplatin for 12 h. Independent experiments were repeated three times and representative data are shown. Scale bar, 20 μm. **e** Quantitative analyses of dead cells. Data are presented as the percentage of PI-staining positive cells. Data are presented as means ± SEM (n = 3 for each group). *p < 0.05, **p < 0.01, ***p < 0.001.

function of PPARδ resulted in decreased cell death induced by cisplatin in cells with overexpression of TAZ (shown in Fig. 9g, h). Together, these results suggest that TAZ acts as a co-repressor of PPARδ restricting GPX4 expression in vitro.

Discussion

In this study, we explored the potential role of TAZ in renal recovery from cisplatin-induced AKI. Using a tubular cell-specific TAZ deletion mouse model, we demonstrated that ablation of TAZ in tubular cells remarkably decreased cisplatin-induced accumulation of lipid peroxidation and ROS production, which were also prevented by ferroptosis inhibitor Fer-1 accompanied by improved renal function and morphological injury. More importantly, our data have shown that in a PPARδ-dependent manner, TAZ consistently reduces the expression of GPX4, thus sensitizing the tubular cells to cisplatin-induced ferroptosis.

The Hippo pathway inhibits YAP/TAZ through phosphorylation, which leads to YAP/TAZ cytoplasmic retention and degradation. The transcriptional factors, YAP and TAZ, primarily bind to enhancer elements using TEAD factors as DNA-binding platforms [10]. YAP and TAZ play some redundant roles in maintaining adult cardiac function, but they may also have different or complementary functions in many situations [31]. Raymond et al. observed increased expression and nuclear translocation of YAP, but not TAZ, in renal proximal tubule epithelial cells in response to AKI, which accelerated renal recovery from IRI [32]. However, a recent report demonstrated that TAZ protects against

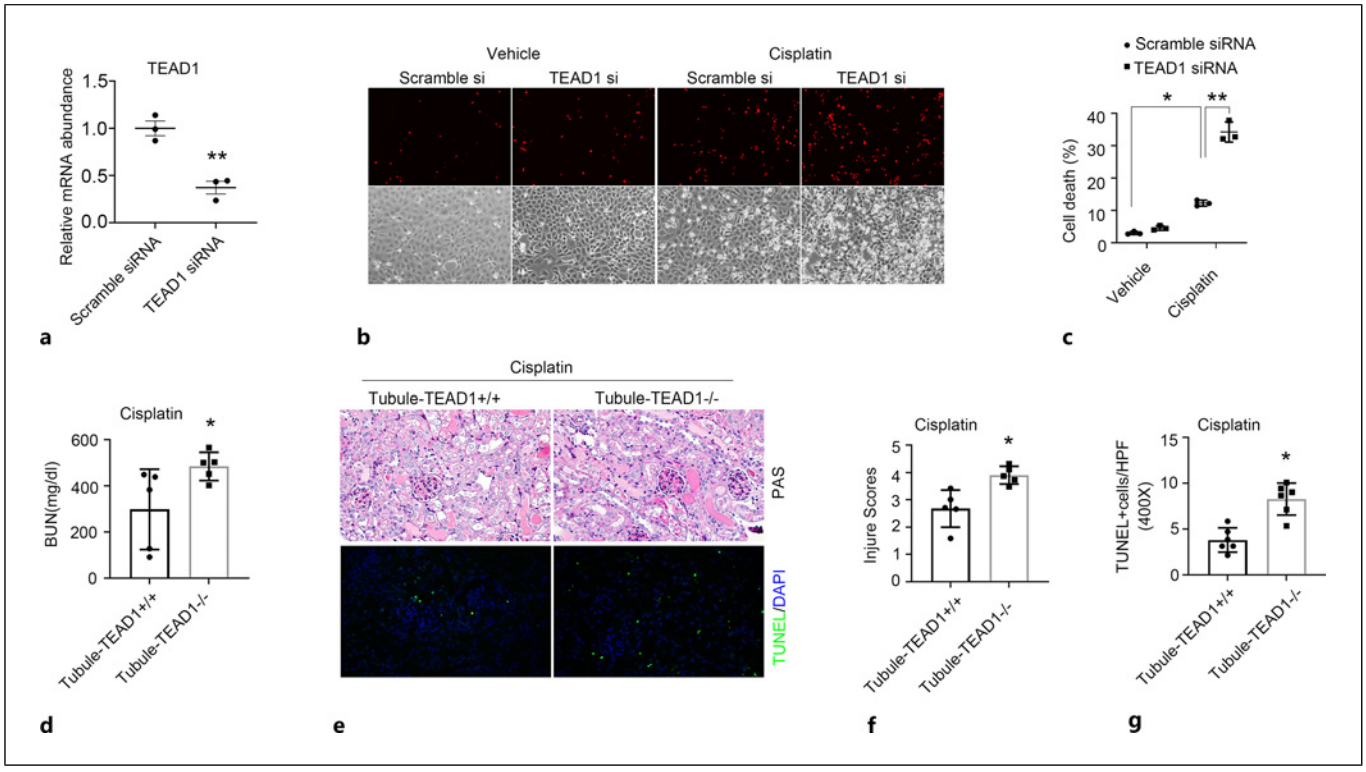


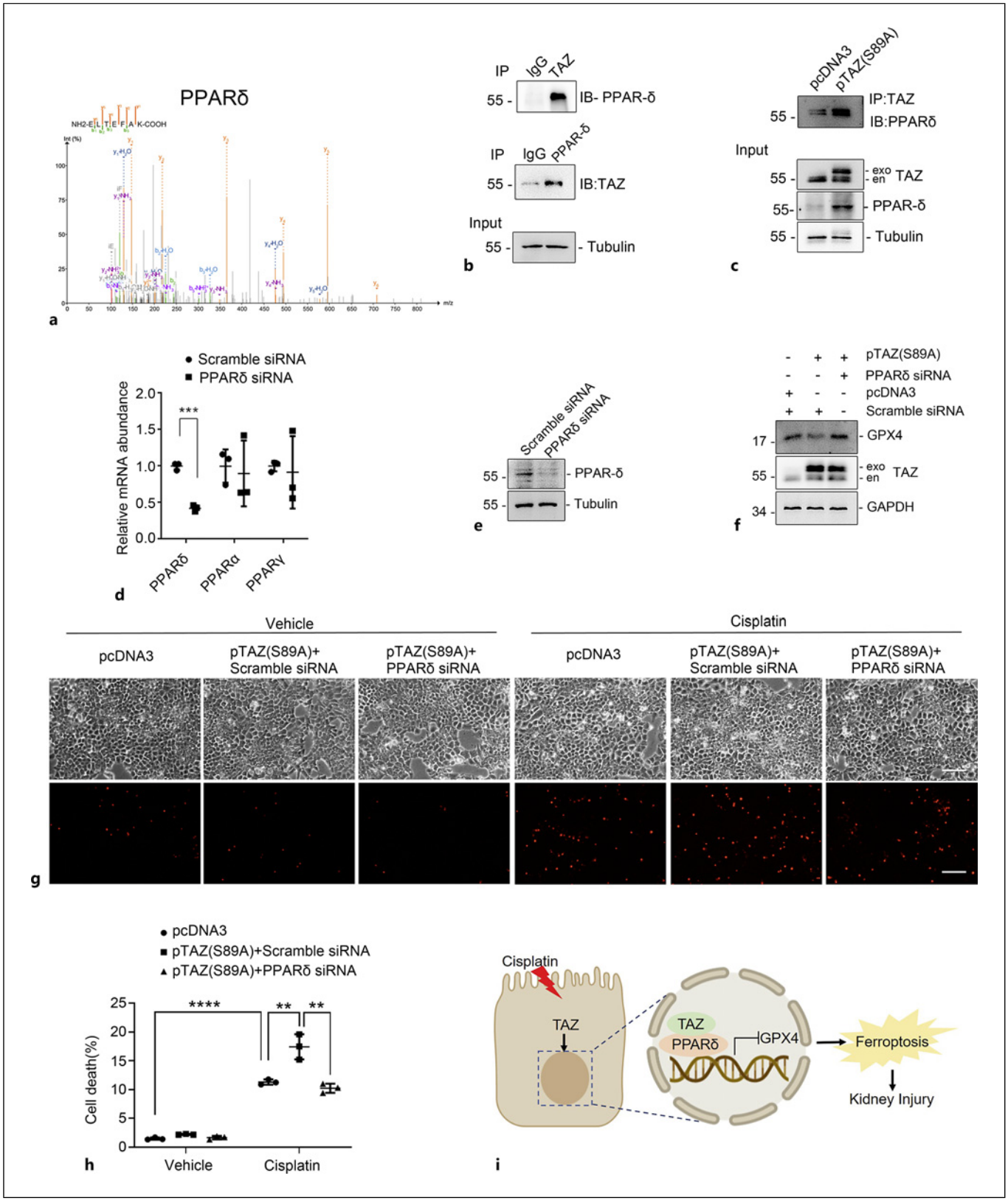
Fig. 8. TEAD is not required for TAZ to regulate tubular cell ferroptosis induced by cisplatin. **a** Real-time qRT-PCR analysis showing the mRNA abundance of TEAD1 as indicated. **b** Phase-contrast images and PI staining showing the NRF-52E cells transfected with TEAD1 siRNA treated with cisplatin for 12 h. Scale bar, 20 μm. **c** Quantitative analysis of dead cells. **d** The graphs showing the BUN level between the Tubule-TEAD1−/− mice and Tubule-TEAD1+/+ littermates on day 3 after cisplatin injection. **e** Representative micrographs showing periodic acid-Schiff (PAS) staining and TUNEL staining. Scale bar, 20 μm. Quantitative analyses of injury scores (**f**) and TUNEL-staining positive cells (**g**) among groups as indicated. Data are presented as means ± SEM (n = 3-6 for each group). *$p < 0.05$, **$p < 0.01$.

ischemic AKI by improving renal function and attenuating tubular apoptosis [33].

In our study, we found that TAZ protein expression and nuclear TAZ localization increased in tubular cells with cisplatin-induced AKI. Conditional deletion of TAZ in tubular cells expedited renal functional recovery and decreased tubular cell death induced by cisplatin. Furthermore, the expression levels of YAP were reduced after cisplatin injection. We speculate that TAZ may play distinct roles that are not redundant with YAP in cisplatin-induced renal toxicity.

AKI is pathologically characterized by renal tubular injury, vascular dysfunction, and inflammation [34, 35]. A variety of particular tubular cell death processes, including apoptosis, necroptosis, and ferroptosis, are recognized as the precipitating factors in AKI [36, 37]. Most of them have been extensively investigated except ferroptosis, possibly due to its discovery recently. Previous publications have shown that ferroptosis is essential in IRI, and oxalate- and folic acid-induced AKI [22, 23].

Recently, two studies reported that ferroptosis is operative in cisplatin-induced AKI. Yashpal et al. [24] observed that various ferroptosis metabolic sensors, including lipid hydro-peroxidation, GPX4 activity, NADPH, and reduced glutathione levels, were operative in cisplatin-induced AKI, which was substantially relieved by Fer-1. Zhang et al. [38] revealed that vitamin D receptor activation protects against cisplatin-induced AKI by inhibiting ferroptosis partly via *trans*-regulation of GPX4. Similarly, in our present work, we observed that cisplatin-induced renal toxicity was substantially relieved by Fer-1. Our in vitro studies showed that cisplatin-induced NRK-52E cell death was attenuated by Fer-1 treatment. Moreover, our studies also showed decreased expression of GPX4 in tubular cells following cisplatin treatment and overexpression of GPX4 attenuated cisplatin-induced cell death. Thus, we



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can conclude that the process of ferroptosis in cisplatin-induced AKI may be modulated by GPX4.

In the past years, the Hippo pathway effectors, YAP/TAZ, have been identified as novel determinants of ferroptosis in cancer cells. In HCT116 cancer cells, higher cell confluence confers resistance to ferroptosis and associated lipid peroxidation, by activating NF2 and the Hippo signaling pathway, along with increased phosphorylation and decreased nuclear localization of YAP [30]. Likewise, TAZ, which is abundantly expressed in renal and ovarian tumors, has been shown to promote ferroptosis by upregulating EMP1-NOX4 and ANGPTL4-NOX2, respectively [26, 27]. Contrarily, YAP/TAZ and ATF4 drive resistance to sorafenib-induced ferroptosis in hepatocellular carcinoma [39]. However, the role of TAZ in the regulation of ferroptosis in cisplatin-induced AKI has not been investigated. Interestingly, in our study, the deletion of TAZ in tubule cells conferred ferroptosis resistance and protected kidneys from cisplatin-induced nephrotoxicity. Overexpression of TAZ exacerbates cisplatin-induced tubular cell ferroptosis in vitro. GPX4, a selenoprotein and one of the three effective systems identified as a potent preventer of ferroptosis, is clearly the best-investigated endogenous inhibitor of ferroptosis [18, 40]. Marcus et al. reported that inhibition of the ferroptosis regulator GPX4 triggers acute renal failure with a complex lipid oxidation signature in mice [20]. In our study, we found that TAZ ablation caused obvious upregulation of the GPX4 expression decreased by cisplatin treatment, while knockdown of GPX4 receded resistance to ferroptosis in TAZ deficient PCTCs. Co-overexpression of GPX4 and TAZ mitigated the cisplatin-induced ferroptotic events when compared to the TAZ overexpression groups. Therefore, TAZ may promote ferroptosis through GPX4.

The TEA domain family of transcription factors, TEADs, is the main transcription factor mediating the biological function of TAZ. Interestingly, we found that ablation of TEAD1 in tubular cells aggravates cisplatin-induced AKI, suggesting that TEAD1 does not mediate TAZ-regulated tubular cell ferroptosis induced by cisplatin. However, the possible mechanism of exacerbating cisplatin-induced kidney injury in TEAD1-knockout mice is unknown. Based on the general understanding of cisplatin-induced kidney injury and the role of TEAD1, we can propose that in the absence of TEAD1, there may be impaired DNA repair, impaired apoptotic pathways, or enhanced inflammation, leading to kidney injury [41–43]. These are speculative explanations, and further studies would be necessary to validate these hypotheses and uncover the actual molecular mechanisms involved in the exacerbation of cisplatin-induced kidney injury in TEAD1-knockout mice.

PPARδ, together with PPARα and PPARγ, are members of the nuclear receptor superfamily of ligand-inducible transcription factors, and they activate their genes through binding to PPAREs as heterodimers with retinoid X receptor (RXR). In the absence of a ligand, PPAR-RXR heterodimers recruit co-repressors, silencing the transcription of target genes [44]. Previously, TAZ, a transcriptional co-regulator, has been shown to function as a transcriptional repressor of PPARγ-induced gene expression, suppressing the PPARγ-mediated adipocyte differentiation program [11]. Concordantly, TAZ was reported to directly binds to PPARγ and function as a co-repressor of PPARγ, contributing to insulin resistance in adipose tissue [13]. However, another study reported that PPARδ binds to YAP1 to promote YAP1 transcriptional activity and progression of gastric cancer progression [45]. Moreover, while the TEAD family has been previously shown to be the major transcription factors cooperate with TAZ to promote specific programs [12, 46], we here show that TEAD1 deficiency in tubular cells aggravates cisplatin-induced AKI, suggesting that TEAD1 is not required for TAZ to regulate tubular cell ferroptosis induced by cisplatin. However, our study reported that TAZ directly bind to PPARδ to restrict GPX4 expression, thereby driving cisplatin-induced ferroptosis. We also observed that the basal

Fig. 9. TAZ and PPARδ collaboratively regulate GPX4 expression and ferroptosis in tubular cells. **a** Cell lysates obtained from NRF-52E cells, with stable transfection of TAZ(S89A), are immunoprecipitated with normal IgG or TAZ antibody. Mass spectrogram of PPARδ. **b** IP assay showing TAZ combining with PPARδ in NRF-52E cells. **c** IP assay showing increased abundance of PPARδ in NRF-52E cells after TAZ(S89A) plasmid transfection. **d** Real-time qRT-PCR analysis showing the mRNA abundance of PPARδ, PPARα, and PPARγ in scramble siRNA and PPARδ siRNA-transfected NRF-52E cells. **e** Western blotting analyses showing the PPARδ expression in scramble siRNA and PPARδ siRNA-transfected cells. **f** Western blotting analyses showing the abundance of GPX4 and TAZ in NRF-52E cells as indicated. **g** Phase-contrast microscopy and PI staining were assayed in indicated groups. Independent experiments were repeated three times and representative data are shown. Scale bar, 20 μm. **h** Quantitative analyses of dead cells as indicated. **i** Working model of this study. Data are presented as the percentage of PI-staining positive cells. Data are presented as means ± SEM (n = 3 for each group). $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

level of GPX4 was not changed in tubular cells after deletion of TAZ compared with that in their counterparts in the absence of cisplatin. Similarly, quantitative mRNA and protein levels of PPARδ were similar in TAZ deficient and TAZ f/f PCTCs, while PPARδ levels were much higher compared to the other two subtypes (data not shown). Thus, we demonstrated the possibility that the transcriptional regulatory activity of TAZ on GPX4 expression is not a linear response, especially in cells with higher basal levels of PPARδ.

In summary, our present work has confirmed the important role of TAZ in cisplatin-induced AKI, while tubular cell TAZ ablation confers ferroptosis resistance and protects kidneys from cisplatin-induced AKI, and this beneficial effect was mediated by regulation of GPX4. Targeting the Hippo pathway or TAZ holds great potential for the treatment of AKI.

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Statement of Ethics

All experiments were performed following the approved guidelines and regulations by the Animal Experimentation Ethics Committee at Nanjing Medical University. All animal experimental protocols were approved by the Institutional Animal Care and Use Committee (IACUC) at Nanjing Medical University (Approval reference No.: IACUC-2103043).

Conflict of Interest Statement

Chunsun Dai is Associate Editor of the journal of "Kidney Diseases." The authors declare that they have no conflicts of interest with the contents of this article.

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Authors Contribution

X.X. preformed the experimental procedures, conception and construction of the study, manuscript writing, and data analysis. X.Z. and L.Z. participated in the in vivo and in vitro experiments. X.S. performed mutant plasmid construction. M.G. and Y.L. contributed to the experimental design and data analysis and performed in vivo animal experiments. M.T., Q.H., and S.D.W. participated in animal model construction. C.D. supervised the entire project, designed the experiment, analyzed the data, revised the manuscript, and approved the final version of the manuscript for publication.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

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