TOPK Activation Exerts Protective Effects on Cisplatin-induced Acute Kidney Injury*

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[Abstract] Objective: T-LAK-cell-originated protein kinase (TOPK), a PSD95-Disc large-ZO1 (PDZ) binding kinase (PBK), is a novel member of the mitogen-activated protein kinase (MAPK) family. Studies have shown that TOPK plays a critical role in the function of tumor cells, including apoptosis and mitosis. However, little is known on the effect of TOPK in cisplatin-induced acute kidney injury (CP-AKI). This study aimed to investigate the role and mechanism of TOPK in CP-AKI.

Methods: Cisplatin was administered to C57BL/6 mice and cultured kidney tubular epithelial cells (TECs) to establish the CP-AKI murine or cellular models. TECs were then stimulated with the specific inhibitor of TOPK OTS514 or transfected with the recombinant-activated plasmid TOPK-T9E to inhibit or activate TOPK. The TECs were treated with AKT inhibitor VIII following stimulation with OTS514 or cisplatin. Western blotting and flow cytometry were used to evaluate the cell cycle and apoptosis of TECs.

Results: The analysis revealed that the TOPK activity was significantly suppressed by cisplatin, both in vivo and in vitro. Furthermore, the pharmacological inhibition of TOPK by OTS514, a specific inhibitor of TOPK, exacerbated the cisplatin-induced cell cycle arrest in the G2/M phase and apoptosis of cultured TECs. Moreover, the TOPK activation via the TOPK-T9E plasmid transfection could partially reverse the cell cycle arrest at the G2/M phase and apoptosis of cisplatin-treated TECs. In addition, AKT/protein kinase B (PKB), as a TOPK target protein, was inhibited by cisplatin in cultured TECs. The pharmaceutical inhibition of AKT further aggravated the apoptosis of TECs induced by cisplatin or TOPK inhibition. TOPK systematically mediated the apoptosis via the AKT pathway in the CP-AKI cell model.

Conclusion: These results indicate that TOPK activation protects against CP-AKI by ameliorating the G2/M cell cycle arrest and cell apoptosis.

Key words: acute kidney injury; cisplatin; cell cycle; apoptosis; T-LAK-cell-originated protein kinase/PSD95-Disc large-ZO1 binding kinase

Acute kidney injury (AKI) is a common clinical problem with high mortality and poor prognosis, and poses a major public health threat. AKI patients require hospitalization, with 50%–70% of these patients requiring intensive care[1]. Cisplatin (CP), as a chemotherapeutic agent, has been extensively used to treat various malignant tumors. However, approximately 30% of CP-treated patients develop renal dysfunction, with AKI being the most common manifestation[2]. An accumulating body of evidence has revealed that the G2/M phase cell cycle arrest of renal tubular epithelial cells (TECs) can trigger the progression of AKI[3] and blunt tubular cell repair, following CP-AKI[4]. In addition, TEC apoptosis plays a vital role in the etiology and pathogenesis of CP-AKI[5,6]. However, the precise pathological mechanism of CP-induced TEC injury via G2/M phase arrest and apoptosis is not fully understood.

T-LAK-cell-originated protein kinase (TOPK), a novel member of the mitogen-activated protein kinase-like serine/threonine kinase (MAPKK) family, is widely expressed in a range of tissues with high levels of proliferation, including tumors, testes and placenta[7–9]. It also plays a critical role in a variety of cellular functions, including the regulation of cellular proliferation[10], autophagy[11], inflammation[12], apoptosis[13], and oxidative stress injury[14]. As a cdc2/cyclin B substrate, TOPK is activated during mitosis via phosphorylation at the Thr 9 residue. The phosphorylation of TOPK promotes G2/M transition, which in turn accelerates the progress of cytokinesis in Hela cells[7]. However, there is little evidence to describe the specific expression or function of TOPK in mammalian kidneys.

Recently, Gao et al reported that remote post-
ischemic treatment exerts antioxidant and anti-inflammatory effects through the activation of TOPK signaling, thereby protecting against renal ischemia/reperfusion injury (IRI)\[^{19}\]. In another study, Netto et al identified a novel loss-of-function TOPK variant (p.Gly43Arg) that could trigger apoptosis, and subsequently contribute to renal epithelial cell damage and kidney stone formation\[^{16}\]. These reports strongly support the fact that TOPK is expressed in mammalian kidneys. CP can increase the apoptosis and cell cycle arrest in TECs, resulting in nephrotoxicity\[^{17, 18}\]. Furthermore, the inactivation of TOPK by 3-deoxys-appanch-alconecan can promote cell cycle arrest at the G2/M phase and apoptosis in colon cancer cell lines\[^{19}\]. A previous study also confirmed that AKT, which is also known as protein kinase B (PKB), is phosphorylated by TOPK at the Ser 473 residue, leading to increased AKT activity, and thereby promoting cell migration and lung cancer\[^{20}\]. Furthermore, a research indicated that the AKT pathway can be activated by dexamethasone to reverse the apoptotic process and protect TECs in CP-AKI\[^{21}\]. Thus, the investigators decided to investigate the function of TOPK in CP-mediated cell cycle arrest and apoptosis in TECs.

The present study aimed to determine the effects of TOPK on CP-AKI by establishing murine models and several cell-cultured models. The protein expression and activity of TOPK were evaluated in the CP-AKI models. Then, the role of TOPK in G2/M cell cycle arrest and apoptosis in CP-treated TECs was investigated. Finally, the role of the TOPK/AKT signaling pathway in the CP-induced apoptosis of TECs was investigated.

1 MATERIALS AND METHODS

1.1 Ethics Statement

The procedures for the animal experiments were ratified by the Ethics Committee of Huazhong University of Science and Technology. The present research was approved by the Institutional Animal Care and Use Committee of Tongji Medical College, Wuhan, China (Ethics number: s2433), and followed the guidelines for the Care and Use of Laboratory Animals by the National Institutes of Health.

1.2 Experimental Protocols for Animals

Eighteen male C57BL/6 mice (age: 8 weeks old; weight: 20–25 g) were purchased from Bionts (China), and were used to establish the CP-AKI model. Mice were housed in an air-filtered, specific pathogen-free environment, with constant temperature and humidity, and a 12-h light/dark cycle. Standard mouse food and water were fed for one week before the administration of saline or CP. Then, these mice were randomly divided into two groups: CP (CP-AKI) and saline (Con) groups \[both solutions were intraperitoneally (i.p.) injected\]. Mice in the CP-AKI group were single-injected i.p. with 27 mg/kg of CP, while the animals in the Con group were single-injected i.p. with an equal volume of saline solution. Then, mice were euthanized on days 0, 2 and 3 after injection. Next, blood samples were collected from all mice to test the level of serum creatinine (Scr) and blood urea nitrogen (BUN) using an Auto-Chemistry Analyzer (Keygentec, China). Then, the kidneys were harvested from each mouse: one kidney for Western blotting and quantitative real-time polymerase chain reaction (RT-qPCR) analysis, and other kidney for hematoxylin and eosin (H&E) staining and immunohistochemistry (IHC).

1.3 Cell Culture and Treatment

Human kidney TECs (HK-2) with normal proximal tubular cell morphological and biochemical features were purchased from the American Type Culture Collection (ATCC, USA). Fifth generation HK-2 cells were used for the subsequent experiments, and no phenotypic changes were observed up to this generation. The cells were cultured in 5% CO\(_2\) at 37°C, and in DMEM supplemented with 10% FBS (Sciencell, USA) in appropriate media, containing 100 mg/mL of streptomycin and 100 U/mL of penicillin (Thermo Fisher Scientific, USA)\[^{22}\].

After seeding to the appropriate confluency, HK-2 cells were administered with 20 µmol/L of CP at four time points (0, 6, 12 and 24 h). The 24-h time point was chosen as the final stimulation time for the subsequent experiments. During the cell experiments, cells were starved of serum for 12 h prior to treatment. Then, the activity of TOPK was inhibited using a specific inhibitor (OTS514; Topscience, China). The same volume of DMSO was used to treat the group of cells without OTS514, as the solvent control. Next, cells were added with 0.01 µmol/L of OTS514 hydrochloride (Topscience, China) for 24 h, as specified for each experiment. The activity of AKT was also inhibited using a specific inhibitor (factor VIII; Topscience, China). Then, the same volume of DMSO was used to treat the group of cells without factor VIII, as the solvent control. Cells were treated with 10 µmol/L of factor VIII-hydrochloride (Topscience, China) for 24 h, as specified for each experiment.

1.4 Cell Transfection

When 70% confluence was reached, the HK-2 cells in the 6-well plate were cultured at 37°C in antibiotic-free medium for 24 h after transfection with pCDNA3.1(+) TOPK-T9E (a recombinant-activated plasmid that contains TOPK, in which Thr9 was mutated to Glu 9; TOPK-T9E group) and pCDNA3.1(+) (the MOCK empty plasmid, MOCK group). Lipofectamine 2000 (Invitrogen, USA) was used as the transfection agent, according to manufacturer’s instructions. Cells were cultured with fresh medium for 4 h after transfection. Then, the TOPK-T9E group and MOCK
group were further divided into two subgroups, and stimulated with or without CP for an additional 24 h. Each experiment was performed in triplicate.

1.5 Hematoxylin-Eosin Staining and Immunohistochemistry

The specimens were fixed in 4% paraformaldehyde, embedded in paraffin, cut into 3-μm thick sections, and stained with H&E. Then, immunohistochemistry (IHC) staining was carried out on the paraffin sections, according to standard procedures. PBS was used to wash the slides. The primary antibodies against p-TOPK Thr 9 (Abcam, UK) and TOPK (Cell Signaling Technology, USA) were used to evaluate the cell apoptosis. The treated or untreated HK-2 cells were cultured to a density of 5×10^4/well in 100 μL of medium in 96-well microplates, and treated with CP at 4 time points (0, 6, 12 and 24 h). Then, 10 μL of CCK-8 reagent was added to each well, according to manufacturer’s instructions, and incubated for another 2 h. The absorbance at 450 nm (A_{450nm}) was measured to indicate the proliferation of cells. All experiments were performed in triplicate. All assays were repeated 8 times, and the wells without cells served as blanks.

1.6 Cell Proliferation Assay

The proliferation was examined using CCK-8 kits (Beyotime, China)\(^{[24]}\). Cells were cultured to a density of 5×10^5/well in 100 μL of medium in 96-well microplates, and treated with CP at 4 time points (0, 6, 12 and 24 h). Then, 10 μL of CCK-8 reagent was added to each well, according to manufacturer’s instructions, and incubated for another 2 h. The absorbance at 450 nm (A_{450nm}) was measured to indicate the proliferation of cells. All experiments were performed in triplicate. All assays were repeated 8 times, and the wells without cells served as blanks.

1.7 Apoptosis and Cell Cycle Assays

A propidium iodide (PI) cell cycle kit (Keygentec, China) was used to investigate the cell cycle, and an Annexin V-FITC/PI apoptosis kit (Keygentec, China) was used to evaluate the cell apoptosis. The treated or transfected HK-2 cells were cultured to a density of 80%–90% in 6-well plates, and collected after trypsin digestion. Then, these cultured cells were washed with PBS, and collected for detection. The apoptosis was detected by re-suspending the cells in 500 μL of binding buffer. Then, a mixture of 5 μL of PI and 5 μL of Annexin V-FITC was added into the binding buffer, and incubated for 30–60 min at room temperature in the dark. Afterwards, the apoptosis was examined using a flow cytometer. Apoptosis in the early stage was indicated by the PI-negative/Annexin V-positive staining, while more advanced apoptosis was indicated by the PI-positive/Annexin V-positive staining. Next, cell cycle assays were performed on cells washed with PBS, and these cells were collected and fixed in 1 mL of ice-cold 70% ethanol overnight at 4°C. Then, these cells were incubated with PI and RNase A (at a ratio of 9:1) at 37°C for 60 min. The cell experiments were repeated in triplicate. The apoptosis data obtained from the flow cytometry were analyzed using the FlowJo V10 software, while the cell cycle-related flow cytometry data were analyzed using the Modfit LT 5.0 software.

1.8 Western Blotting

The HK-2 cells and kidney tissues were lysed with radioimmunoprecipitation assay (RIPA) lysis buffer (Beyotime, China), according to standard procedures. Then, the protein concentration was calculated using a BCA protein assay kit (Thermo Scientific, USA). Afterwards, the proteins were separated using SDS-polyacrylamide gel (8%–12%), transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore Corp., USA), and blocked by 5% skimmed milk (Beyotime, China) for 2 h. Next, the membranes were incubated with primary antibodies raised against the following: TOPK (Santa Cruz, USA); p-TOPK, NGAL (Abcam, USA); α-tubulin (Proteintech, USA); Aurora B, Bcl2, Bax, p-AKT and AKT (Cell Signaling Technology, USA).

1.9 Total RNA Extraction, Reverse Transcription and Quantitative Real-time Polymerase Chain Reaction

According to manufacturer’s protocol, the total RNA was extracted from cultured HK-2 cells and the kidney cortex tissue using TRIzol reagent (Novizan, China). A total of 1 μg of RNA was reversely transcribed in a volume of 20 μL using the PrimeScript RT Reagent Kit (Novizan, China). Then, quantitative real-time polymerase chain reaction (RT-qPCR) was performed through the StepOnePlus Real-Time PCR System (Applied Biosystems, USA) using SYBR Premix Ex Taq II (Novizan, China). The RT-qPCR protocol was as follows: pre-denaturation at 95°C for 10 min, denaturation at 95°C for 15 s, and annealing at 60°C for 15 s, with a total of 40 cycles. The sequences of the specific primers for the polymerase chain reaction amplification were synthesized, as shown in table 1. Three independent experiments were performed. GAPDH was used as the control housekeeping gene. The data obtained from the RT-qPCR were analyzed using the algorithm of the 2^(-ΔΔCt) method.

1.10 Statistical Analysis

All experiments were repeated in triplicate. The statistical analysis was performed using SPSS version 20.0, and all data were presented as mean±standard deviation (SD). Student’s unpaired t-test was used for comparing two groups, while one-way analysis of
2 RESULTS

2.1 TOPK Was Inhibited in CP-AKI In Vivo and In Vitro

In order to explore whether TOPK is involved in CP-induced renal tubular dysfunction, an AKI murine model was initially established by CP injection i.p., and this was subsequently used to detect the protein levels of TOPK and p-TOPK. After 2 or 3 days of CP injection, the serum Scr levels and BUN significantly increased, with higher levels on day 3 than that on day 2 (fig. 1A). Consistent with these changes, the H&E staining score revealed increased severe tubular damage in the day 3 group, when compared to the day 2 group. This was evidenced by the tubular casts, tubular dilatation, tubular cell death, and loss of brush border (fig. 1B). Thus, the time point of 3 days was selected for the subsequent experiments. Concurrent with the pathological changes in kidney tissues, the mRNA levels of kidney injury molecule-1 (KIM-1)[25] and monocyte chemoattractant protein-1 (MCP-1)[26], which are well-characterized biomarkers (KIM-1)[25] and monocyte chemoattractant protein-1 (MCP-1)[26], were evaluated, and the Western blotting revealed a significant increase in NGAL (a specific protein biomarker of AKI) and a marked increases (fig. 1C). Furthermore, the NGAL protein levels also sharply increased, which corresponded to the induction of CP-AKI in vitro (fig. 1G). In addition, the TOPK protein expression remained constant, but the p-TOPK levels dramatically decreased in a time-dependent manner following the CP treatment (fig. 1G). These data indicate that TOPK plays a crucial role in CP-AKI in vivo and in vitro.

2.2 Cisplatin Suppressed the Proliferation and Promoted Cell Apoptosis in Cultured HK-2 Cells

In order to investigate whether the proliferation and apoptosis of HK-2 cells subjected to CP are associated with the downregulation of p-TOPK (fig. 1E), the same 4 CP-stimulated time points (0, 6, 12 and 24 h) were selected for the HK-2 cells. Initially, cell proliferation was detected using the CCK-8 kit, and it was found that the cell proliferation significantly declined after treatment with CP for 24 h (fig. 2A). Then, the Aurora B levels were evaluated, and the Western blotting revealed a dramatic reduction in Aurora B in a time-dependent manner after stimulation with CP for 6–24 h (fig. 2B). Hence, Western blotting was used to evaluate the expression levels of anti-apoptotic protein Bcl2 and pro-apoptotic protein Bax. The Western blotting revealed that the Bcl2/Bax ratio significantly declined after 24 h of CP treatment (fig. 2C). Collectively, these results suggest that TOPK may alter the HK-2 proliferation by regulating the cell cycle progression, and simultaneously, TOPK may be involved in the cellular apoptosis of CP-treated TECs.

2.3 TOPK Was Involved in Cisplatin-mediated G2/M Arrest in Cultured HK-2 Cells

Cell proliferation significantly decreased after 24 h of CP stimulation, which was probably due to the delay of cell cycle progression[27, 28]. In order to clarify the effect of TOPK on the cell cycle of CP-treated TECs, 24 h was selected as the appropriate duration of CP stimulation for the subsequent studies. Initially, the TOPK activity was inhibited with its specific inhibitor, OTS514. As shown in the Western blot results, the OTS514 treatment groups had critically decreased levels of p-TOPK, when compared to the groups without OTS514 treatment (fig. 3A). This demonstrates the successful inhibition of TOPK. Then, Aurora B was detected, and the Western blotting indicated that OTS514 induced a further reduction in Aurora B after the CP treatment (fig. 3A) via the
inhibition of TOPK, and the more suppression of the Aurora B expression induced by OTS514 treatment. The flow cytometry revealed the significant increase in G2/M fraction of HK-2 cells treated with OTS514 and CP, when compared to cells stimulated with CP only. This indicates that the inhibition of TOPK can induce additional G2/M phase accumulation in CP-AKI (fig. 3B). Next, a constitutively TOPK-activated T9E mutant plasmid (TOPK-T9E) was transfected to activate TOPK, and this manifested through the increase in TOPK protein expression (fig. 3C). The reduction in Aurora B induced by CP was partially reversed by the T9E transfection (fig. 3C). Similarly, T9E reduced the cell cycle arrest in the G2/M phase (fig. 3D). These
data indicate that the promotion of G2/M transition by TOPK may be partly mediated by Aurora B.

2.4 TOPK Played a Role in Cisplatin-mediated Cell Apoptosis in Cultured HK-2 Cells

Apoptosis, as a major form of cell death, is involved in the pathogenesis of CP-AKI. Studies have revealed that TOPK is also involved in apoptosis via multiple signaling pathways\cite{13, 14}. Hence, the investigators aimed to explore the role of TOPK in CP-mediated apoptosis in HK-2 cells. Initially, the Bcl2/Bax ratio was detected by Western blotting. It was found that the OTS514 groups had a lower Bcl2/Bax ratio than the groups without OTS514 treatment (fig. 4A). The flow cytometry further confirmed that OTS514 increased the levels of apoptosis in CP-AKI (fig. 4B). Next, the effect of TOPK activation in CP-mediated AKI was investigated. HK-2 cells were transfected with the TOPK-T9E or MOCK plasmid, and incubated with or without CP. It was found that the Bcl2/Bax ratio declined in the CP groups, but this was partially reversed by the activation of TOPK (fig. 4C). The role of the TOPK activity in apoptosis was verified by flow cytometry (fig. 4D). This confirms that the activation of TOPK can partially improve the cellular apoptosis in CP-AKI.

2.5 TOPK Regulated AKT Signaling Pathway in HK-2 Cell Injury Stimulated by Cisplatin

AKT is the downstream protein of TOPK, and is involved in CP-induced apoptosis after TEC injury. In order to explore the mechanism by which TOPK regulates CP-induced injury in TECs, the activity of AKT was evaluated. Western blot results also showed that CP can inhibit the phosphorylation of AKT in cultured HK-2 cells (fig. 5A). The decrease in p-AKT induced by CP was aggravated by the inhibition of TOPK, or reversed by the activation of TOPK (fig. 5A and 5B). These results imply that TOPK may regulate CP-induced HK-2 cell injury by targeting the AKT signaling pathway.

2.6 TOPK/AKT Signaling Pathway Plays a Key Role in Cisplatin-induced HK-2 Cell Apoptosis

In order to further clarify whether the TOPK/AKT pathway is involved in the CP-induced apoptosis of HK-2 cells, HK-2 cells were treated with factor VIII, which is a specific inhibitor of AKT. The Western blot results revealed that factor VIII further reduced the phosphorylation of AKT after CP treatment (fig. 6A). Meanwhile, factor VIII reduced the Bcl2/Bax ratio, indicating that the apoptosis of HK-2 cells stimulated by factor VIII more significantly increased after CP and OTS514 treatment (fig. 6B). Furthermore, the flow cytometry revealed that factor VIII markedly aggravated the apoptosis of HK-2 cells subjected to CP or OTS514 stimulation (fig. 6C). These results suggest that TOPK activation exerts an anti-apoptotic function via the AKT signaling pathway in TEC injury induced by CP treatment.

3 DISCUSSION

The present study established a mouse model and several cell-cultured models to investigate the function of TOPK protein, in terms of cell cycle arrest and apoptosis in CP-AKI. Several novel discoveries were made. First, TOPK exhibited a high activity in normal renal tubules, but exhibited a markedly low activity in CP-AKI. Second, the pharmacological inhibition of TOPK obviously promoted G2/M arrest and accelerated the cell apoptosis in CP-AKI in vitro. Third, the consistent activation of TOPK partially reversed the cell cycle arrest and apoptosis after CP treatment in HK-2 cells. Fourth, the mechanistic studies demonstrated that the inhibition of TOPK can aggravate the G2/M phase arrest, but this might be partially mediated by Aurora B. Furthermore, the CP-induced apoptosis was partially mediated by the inhibition of the TOPK/AKT signaling pathway. Focus was given on the effects of TOPK on CP-AKI, in order to
provide novel and considerable insights for the therapy of CP-AKI (fig. 7).

TOPK has been reported to be abundantly expressed in tumor cell lines, but few studies have described the expression of TOPK in kidney tissues [29]. Gao et al. [15] and Nettuwakul et al. [16] both reported that TOPK is involved in certain pathophysiological states in the kidneys, but they did not describe the detailed function in kidney diseases. In the present study, it was observed that the expression of p-TOPK declined in CP-treated TECs, both in vivo and in vitro.

However, the expression of the TOPK protein under in vivo conditions was slightly higher than that under in vitro conditions, in the AKI models (fig. 1D and 1G).
TOPK plays a role in cisplatin-mediated cell apoptosis in cultured HK-2 cells

A: Bcl2 and Bax in HK-2 cells treated with cisplatin (CP) or OTS514 were measured by Western blotting. B: Bcl2 and Bax in HK-2 cells stimulated with CP or transfected with TOPK-T9E were measured by Western blotting. C: Cell apoptosis in HK-2 cells after treatment with CP or OTS514 was detected by flow cytometry. D: Cell apoptosis in HK-2 cells added with CP or transfected with TOPK-T9E was detected by flow cytometry. 

Fig. 4 TOPK plays a role in cisplatin-mediated cell apoptosis in cultured HK-2 cells

A: Bcl2 and Bax in HK-2 cells treated with cisplatin (CP) or OTS514 were measured by Western blotting. B: Bcl2 and Bax in HK-2 cells stimulated with CP or transfected with TOPK-T9E were measured by Western blotting. C: Cell apoptosis in HK-2 cells after treatment with CP or OTS514 was detected by flow cytometry. D: Cell apoptosis in HK-2 cells added with CP or transfected with TOPK-T9E was detected by flow cytometry. 

Fig. 5 TOPK regulates the AKT signaling pathway in cisplatin-treated HK-2 cells

A: AKT and p-AKT in HK-2 cells treated with cisplatin (CP) or OTS514 were measured by Western blotting. B: AKT and p-AKT in HK-2 cells treated with CP or transfected with TOPK-T9E were measured by Western blotting. 

Consequently, it was found that there was an abundance of non-specific TOPK staining in the tubular lumen, as presented in fig. 1E. This was particularly associated with the proteinaceous casts in the AKI mouse model. It was considered that the enhanced TOPK expression detected by immunoblotting may have been caused by non-specific binding. The constant TOPK protein expression level in CP-stimulated HK-2 cells reinforce the fact that CP treatment may not affect the protein synthesis or degradation of TOPK. These results show that TOPK plays a physiological role in CP-AKI. TOPK was activated in the ischemia-reperfusion
AKI (IR-AKI) mouse model[35]. However, it was found that the activity of TOPK decreased in CP-AKI mouse kidneys. Although TOPK exhibited opposite activity states in IR-AKI and CP-AKI, these results confirm the renoprotection effect of TOPK on AKI. The inconsistent expression of p-TOPK may have been mediated by different signaling pathways. TOPK is the downstream target protein for cyclinB1/cdk1, and it can be phosphorylated by the ATM/cdc25 signaling pathway[30–32]. Previous reports have revealed that ATM exhibits different levels of activity when comparison was performed between CP-AKI and IR-AKI: ATM is activated in a time-dependent manner in IR-AKI[33], but this can only be activated in the early stages of CP-AKI, and this is subsequently inactivated and cleaved to facilitate the apoptosis during the latter phases of CP-AKI[34]. Therefore, when comparison was performed between CP-AKI and IR-AKI, the differential activity of ATM may be responsible for the variable levels of TOPK. In the future, more extensive research needs to be carried out to clarify the precise role of TOPK in CP-AKI, and determine the differences between the activated pathways observed in CP-AKI and IR-AKI.

Study has shown that TOPK is correlated to the cell cycle arrest at G2/M in promyelocytes[34]. Yang et al demonstrated that TECs that present with G2/M arrest are impaired in repair, and contribute to the release of profibrotic cytokines, including TGFβ-1 and connective tissue growth factor (CTGF), thereby inducing interstitial fibrosis in toxic, obstructive and ischemic models of AKI[34]. Consistent with these reports, the present results suggest that CP treatment can lead to cell cycle arrest at the G2/M phase in HK-2 cells. However, a recent study suggested that cell cycle G2/M arrest may not exist in a moderate injury AKI model[35]. The different observations regarding the
G2/M arrest in AKI may be due to differences in the degree or time of kidney injury. Thus, the present data implies that TOPK may potentially affect the renal tubule fibrosis. However, this still needs to be further explored in the future.

Aurora B, which is a member of the Aurora Kinase family, is essential for cell division, and induces G2/M arrest and hyperploidy[27]. Recent studies have demonstrated that the deletion or inhibition of Aurora B prompts the G2/M phase arrest[36–38]. As a mitotic kinase, TOPK is also critical in the cell cycle. Aurora B kinase suppression and TOPK kinase inhibition can block mitosis, causing G2/M arrest[39–41]. Consistent with these studies, the present data revealed that the inhibition of TOPK resulted in the extreme decrease of Aurora B, and the pronounced increase in cell cycle arrest at the G2/M phase. However, the activation of TOPK partially reversed the reduced Aurora B and G2/M arrest induced by CP treatment. This indicates that Aurora B kinase is the downstream of TOPK, and that the inhibition of TOPK in CP-AKI exacerbates the G2/M phase arrest, which may be partially mediated by Aurora B. Interestingly, Xie et al reported that the overexpression of AKT can reverse the inhibition of Aurora B activity and cell proliferation mediated by bufalin treatment in human colon cancer HT-29 cells[42]. On the other hand, the knockdown of Aurora B can suppress the PI3K-AKT pathway, thereby inhibiting the invasion and migration of lung and osteosarcoma cells[43, 44]. Furthermore, the regulation between Aurora B and AKT may be reciprocal. Since the direct evidence that associated AKT with CP-induced G2/M arrest in AKI was not clarified, further investigations are needed to determine whether AKT affects the G2/M arrest in CP-AKI. Moreover, the AKT signaling pathway can exert renoprotective effects[45]. In the present study, it was demonstrated that AKT inhibitor factor VIII can aggravate the apoptosis induced by OTS514 and CP in HK-2 cells. This suggests that CP induces apoptosis in HK-2 cells by inhibiting the TOPK/AKT pathway.

In conclusion, the present study is the first to provide data that demonstrates TOPK functions as a novel survival signal by reducing the cell cycle arrest in the G2/M phase, and alleviating the apoptosis of TECs in CP-AKI. In addition, it was found that crucial cell cycle regulator kinase Aurora B is the downstream of TOPK, and that the TOPK promotion of G2/M transition may be mediated by Aurora B, to some extent. These present results suggest that the anti-apoptotic effect of TOPK can be achieved via the downstream AKT signaling pathway. However, since this involves a complex regulatory network, there is still a need to activate TOPK in vivo, and explore other downstream signaling pathways of TOPK in CP-AKI in the future. The present data demonstrates that targeting TOPK may represent a novel therapeutic strategy for the prevention of CP-AKI.

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Conflict of Interest Statement
On behalf of all authors, the corresponding author states that there is no conflict of interest.

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