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Autophagy Inhibition Sensitizes Renal Tubular Epithelial Cell to G1 Arrest Induced by Transforming Growth Factor beta (TGF-β)

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Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

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Background: Cell cycle arrest and autophagy have been demonstrated to be involved in various transforming growth factor (TGF)-β-mediated phenotype alterations of tubular epithelial cells (TECs) and tubulointerstitial fibrosis. But the relationship between cell cycle arrest and the autophagy induced by TGF-β has not been explored well.

Material/Methods: The effects of autophagy inhibition on TGF-β-induced cell cycle arrest in TECs were explored *in vitro*. Human kidney-2 (HK-2) cells were stimulated by TGF-β with or without a combined treatment of autophagy inhibitor chloroquine (CQ) or bafilomycin A1 (Baf).

Results: Autophagy inhibition by CQ or Baf promotes the suppression of growth in TGF-β-treated HK-2 cells, as detected by the Cell Counting Kit-8 (CCK-8) method. In addition, CQ or Baf stimulation enhances G1 arrest in TGF-β treated HK-2 cells, as investigated using propidium iodide (PI) staining and flow cytometry, which was further confirmed by a decrease in the expression of phosphorylated retinoblastoma protein (p-RB) and cyclin-dependent kinase 4 (CDK4). The upregulation of p21 induced by CQ or Baf may mediate an enhanced G1 arrest in TGF-β treated HK-2 cells. Western blot analysis showed that TGF-β-induced expression of extracellular matrix fibronectin was notably upregulated in the presence of autophagy inhibitors.

Conclusions: Inhibition of autophagy sensitizes the TECs to G1 arrest and proliferation suppression induced by TGF-β that contributes to the induction of tubulointerstitial fibrosis.

MeSH Keywords: Autophagy • Epithelial Cells • Fibrosis • G1 Phase Cell Cycle Checkpoints • Transforming Growth Factor beta

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Background

Renal fibrosis, characterized by glomerulosclerosis and tubulointerstitial fibrosis, is the final common pathological feature of various chronic kidney diseases (CKD) [1,2]. Although many kidney diseases are initiated by glomerular injuries, tubulointerstitial fibrosis is termed so, due to excessive accumulation and deposition of extracellular matrix (ECM) within the tubule-interstitium; and is predominant to glomerulosclerosis in causing a renal function decline [3–5]. Renal tubulointerstitial intrinsic cells (tubular epithelial cells [TECs] and fibroblasts) and infiltrated inflammatory cells (such as macrophages and T cells) interact with each other and codetermine the development and progression of tubulointerstitial fibrosis [6]. A growing evidence shows that renal TECs not only act as a victim, but also play a crucial role during the tubulointerstitial injury by producing various proinflammatory and profibrotic cytokines, in particular, transforming growth factor-β (TGF-β) [7,8]. In Smad-dependent and -independent pathway, TGF-β produced by injured TECs not only stimulates fibroblasts that transform into myofibroblasts phenotype that can synthesize extracellular matrix, but also induce TECs to undergo a partial epithelial-mesenchymal transition and dedifferentiate to secrete excessive platelet-derived growth factors and connective tissue growth factors as well as TGF-βs that promote fibrogenesis [9]. However, our knowledge about the mechanisms of TGF-β-mediated tubulointerstitial fibrosis is very limited.

Despite TECs being sensitive to the injury and yet a participant to tubulointerstitial injury, they also have a notable regenerative capacity and contribute to renal repair [10]. Ischemia and drug-induced renal injury causes severe injured TECs to undergo necrosis and apoptosis [11,12], but the remaining TECs re-enter the cell cycle, replace the lost TECs, and restore the renal function through dedifferentiation, proliferation, and redifferentiation [13]. However, maladaptive repair happens when the insult is severe or persistent, and TECs fail to get through the cell cycle and complete cell division [14]. Thus, a correlation exists between an increased proportion of TECs arrested in the cell cycle and a sustained kidney injury and fibrotic progress [14]. TECs arrested in the G1/S or G2/M increase the expression of TGF-β and promote tubulointerstitial lesions [15,16]. Interestingly, TGF-β itself can induce both G1/S [17] and G2/M [18] arrest in TECs, and targeting TGF-β pathway by Smad7 prevents TECs from G1/S arrest and protects them against the ischemia-reperfusion-induced acute kidney injury [19].

Autophagy, an evolutionarily conserved cellular behavior, maintains the cellular homeostasis by destroying the damaged cell organelles and long-life proteins and recycling the degradative compounds [20]. Autophagy dysfunction is often associated with fibrotic kidney diseases [20,21]. But the role of autophagy in tubulointerstitial fibrosis is complex and inconsistent. Persistent expression of the TGF-β-induced autophagy and dedifferentiation in renal tubules, contributes to subsequent tubulointerstitial fibrosis [22,23]. In addition, autophagy seems to negatively regulate the TGF-β signaling, partially via degradation of TGF-β [24], and induced type-I collagen [25].

Although the cell cycle arrest and autophagy have been demonstrated to be involved in TGF-β-mediated alterations of TECs phenotype and tubulointerstitial fibrosis, the relationship between cell cycle arrest and autophagy induced by TGF-β has not been well explored. In the present study, we attempted to identify the functional role of autophagy in the TGF-β-induced cell cycle arrest of TECs. This work may facilitate the discovery of novel therapeutic strategies for the treatment of tubulointerstitial fibrosis via targeting autophagy.

Material and Methods

Reagents and antibodies

For cell culture, the Dulbecco's Modified Eagle Medium (DMEM) medium (C11995500BT), fetal bovine serum (10270106) and penicillin-streptomycin (15140122) were obtained from Gibco (New York, NY, USA). Recombinant TGF-β1 (rTGF-β1, 240-B-002) was procured from R&D systems (Minneapolis, MN, USA), while chloroquine (CQ, C6628) and bafilomycin A1 (Baf, 196000) were obtained from Sigma-Aldrich (St Louis, MO, USA). Cell Counting Kit-8 (C0037) and propidium iodide (PI) (ST511) were purchased from Beyotime Biotechnology (Shanghai, China). For western blotting analysis, the antibodies against p62/sequestosome 1 (ab56416), p16 (ab51243), fibronectin (ab23750), collagen I (ab34710), cyclin-dependent kinase 4 (CDK4, ab137675), CDK6 (ab151247), cyclin D1 (ab16663), tubulin (Ab59680), β-actin (ab8227), and α-smooth muscle actin (α-SMA) were purchased from Abcam (Cambridge, MA, USA). Antibodies to CDK2 (2546S), phosphorylated CDK-2 (2561S), phosphorylated retinoblastoma protein (p-RB, 8516), cyclin A (4656S), and p21 (2947S) were obtained from Cell Signaling (Danvers, MA, USA). Antibodies to cyclin E (SAB4503516) and microtubule-associated protein 1 light chain 3B (LC3, L7543) were purchased from Sigma-Aldrich. GAPDH antibody (abs132004) was obtained from Absin Bioscience Inc. (Shanghai, China). Horseradish peroxidase (HRP)-conjugated anti-mouse IgG (A0216), anti-rabbit IgG (A0208), and anti-goat IgG (A0181) were purchased from Beyotime as secondary antibodies. Clarity™ Western ECL Substrate (170-5060) was procured from BioRad (Hercules, CA, USA). RIPA lysis buffer (P0013E) and phenylmethanesulfonyl fluoride (PMSF, ST506) were obtained from Beyotime. BCA Protein Assay Kit (23225) was acquired from Pierce (Rockford, IL, USA). Phosphatase inhibitor cocktail (P1260) was obtained from Applygen (Beijing, China).

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Cell culture

Human kidney tubular epithelial cells (HK-2) were obtained from ATCC (CRL-2190™, Manassas, VA, USA) and maintained in a completed medium (DMEM with 10% fetal bovine serum and 1% penicillin-streptomycin) at 37°C with 5% CO_2. 2 ng/mL rTGF-β1 was used to stimulate cells *in vitro*. For chemical inhibition of autophagy, HK-2 cells were treated with 10 μM CQ or 100 nM bafilomycin A1 (Baf) for 24 hours with or without TGF-β1 stimulation.

Cell proliferation analysis

Cell Counting Kit-8 (CCK-8) was used for the detection of cell proliferation in this study according to the manufacturer's instructions. The absorbance at 450 nm was measured by a microplate reader (BioTek, ELx800, Winooski, VT, USA).

Cell cycle analysis

The cell cycle analysis was performed by propidium iodide (PI) DNA staining and subsequent flow cytometry. Briefly, harvested HK-2 cells were fixed in cold 70% ethanol for 30 minutes at 4°C. After being washed with phosphate-buffered saline (PBS) and incubated with RNase, the cells were stained in PI staining solution and then analyzed by FACSCanto II platform (BD, FACSCanto II, San Jose, CA, USA).

Western blotting analysis

Proteins from the cultured cells were extracted using radioimmunoprecipitation assay (RIPA) buffer added with a protease inhibitor PMSF (phenylmethylsulfonyl fluoride) and phosphatase inhibitor cocktail. Lysates were centrifuged at 13 000×g at 4°C for 15 minutes, and the supernatants were collected. The concentration of proteins was detected by the BCA Protein Array kit. An equal amount of protein samples were treated with 12% sodium dodecyl sulfate (SDS)-polyacrylamide gels and transferred on to polyvinylidene fluoride (PVDF) membranes (EMD Millipore, ISEQ00010). After blocking, the membranes were incubated with diluted primary antibody overnight at 4°C and incubated with HRP-conjugated secondary antibody for another 2 hours. After washing, the membranes were incubated with an enhanced chemiluminescence (ECL) substrate. The signals were detected by the Azure C500 Western Blot Imaging System (Azure Biosystems, Dublin, CA, USA) and then were quantified by using the ImageJ software (NIH).

Statistical analysis

All data were obtained from at least 3 independent experiments. Data were presented as mean±standard error of the mean (SEM). For comparisons among multiple groups, one-way analysis of variance (ANOVA) was used followed by Tukey's *post hoc* tests. $P<0.05$ was determined as a statistically significant difference in this study. Data analysis and graphics were performed using GraphPad Prism 5 (GraphPad Software, San Diego, CA, USA).

Results

Inhibition of autophagy promoted growth arrest in TGF-β treated HK-2 cells

First, we tested the suppressive effects of CQ and Baf on TGF-β-induced autophagy in HK-2 cells. As shown in Figure 1, in contrast to the control group cells, TGF-β slightly induced autophagy activation in HK-2 cells as evidenced by a little increase in the expression of autophagic marker LC3-II detected by western blotting. As expected, HK-2 cells treated with Baf or CQ notably induced the accumulation of LC3-II and p62 upon exposure to TGF-β. These data indicated that Baf and CQ treatment efficiently blocked the autophagy flux induced by TGF-β in HK-2 cells.

Then, we detected the proliferation capacity of HK-2 cells in the 4 groups by CCK-8 method that based on the reduction of WST-8, a highly water-soluble tetrazolium salt, to a water-soluble orange colored formazan dye by intracellular dehydrogenase activities. Directly, the live cell counting is proportional correlation with the amount of the formazan dye generation. As shown in Figure 2, TGF-β administration inhibited the proliferation capacity of HK-2 cells at 24 and 48 hours. In addition, a combination treatment with TGF-β and autophagy inhibitors (Baf or CQ) further markedly suppressed the HK-2 cell proliferation, which suggested that inhibition of autophagy promoted growth arrest in TGF-β-treated HK-2 cells.

Inhibition of autophagy enhanced G1 arrest in TGF-β treated HK-2 cells

Since cell proliferation is tightly negatively regulated by cell cycle arrest, we then analyzed the effect of autophagy inhibition on cell cycle. It is well known that the normal process of cell division requires that the parent cell must replicate its DNA faithfully in order that a full copy of the genetic information can be received by each daughter cell. In the interphase of cell cycle, the period before the synthesis of DNA represents G1 phase, the period during which the DNA content increases as new DNA synthesis refers to S phase and the period after DNA synthesis has occurred but prior to the start of mitosis is known as G2 phase. As such, there have different DNA contents in different stages of the cell cycle, which can be detected by staining cells with PI, a fluorescent molecule that intercalates with DNA at a specific ratio, using flow cytometry

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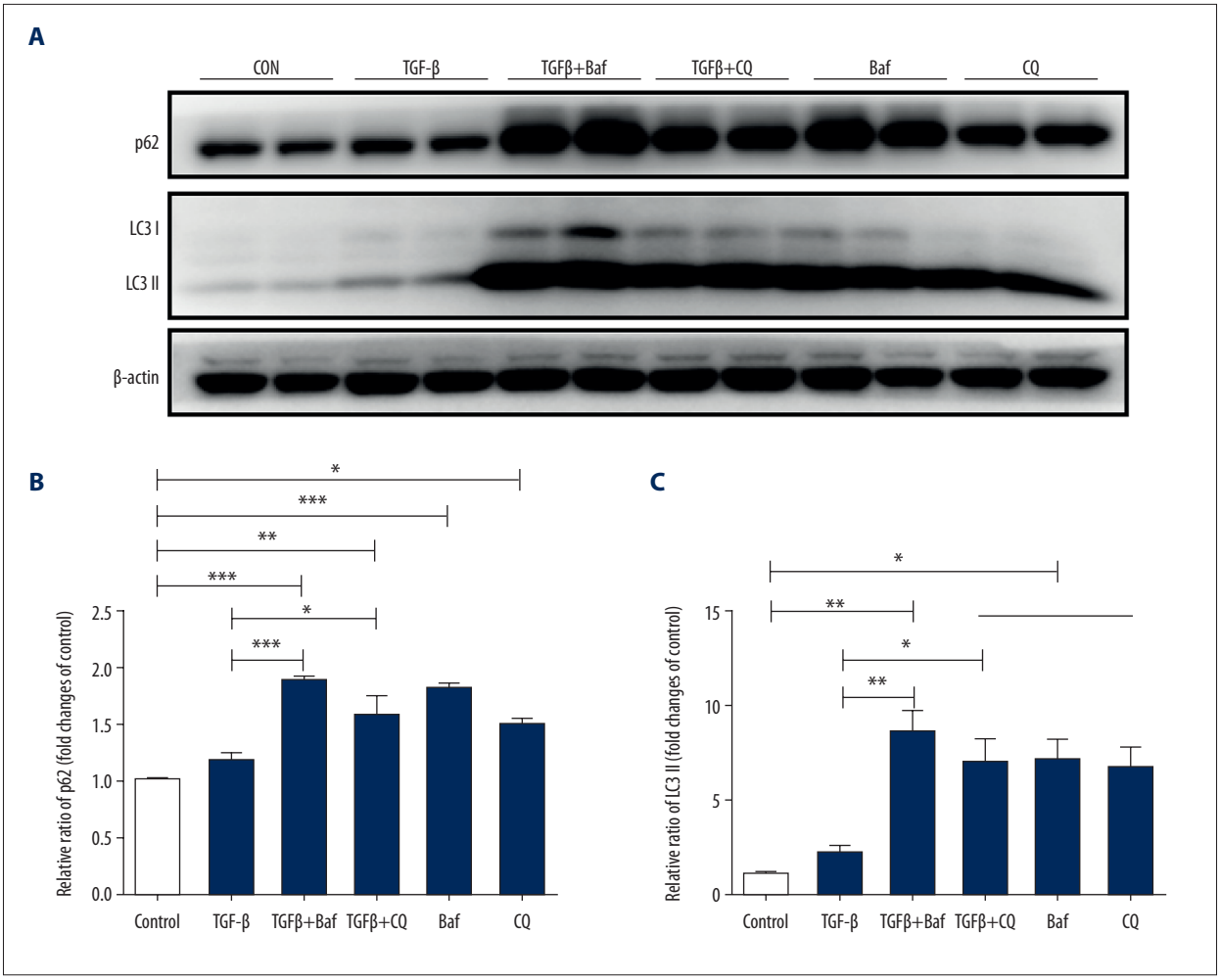

Figure 1. Expression of autophagy-related protein LC3 and p62 in HK-2 cells as detected by western blotting. (**A**) Representative blots of LC3 and p62 expression levels in HK-2 cells. (**B**) Quantitation of p62 expression. (**C**) Quantitation of LC3-II expression. Data represent the mean±SEM for at least 3 independent experiments. * $P<0.05$, ** $P<0.01$, *** $P<0.001$ between 2 groups. HK-2 – human kidney-2; SEM – standard error of the mean; TGF – transforming growth factor; Baf – bafilomycin A1; CQ – chloroquine; CON – control.

analysis. Therefore, the intracellular PI fluorescence level is directly proportional to the percentage of cells in each phase of the cell cycle. As shown in Figure 3, when subjected to TGF-β stimulation, a proportion of HK-2 cells in the G1 phase were elevated but the ratio of HK-2 cells in S and G2/M phase was reduced. Furthermore, the proportion of TGF-β-treated HK-2 cells in the G1 phase was significantly upregulated when co-stimulated with Baf and CQ.

Inhibition of autophagy affected the level of proteins involved in cell cycle regulation in TGF-β treated HK-2 cells

The balance between cell cycle arrest and cell proliferation are controlled by cyclin-dependent kinase network, and our results indicated that the inhibition of autophagy enhanced G1 arrest in TGF-β treated HK-2 cells, so we next detected the expression of master regulators in G1/S transition. It is well accepted that the transition of the first gap phase into the S phase is initially driven by CDK4/CDK6 and their regulator cyclin D1, and later by cyclin E and CDK2 complex. Activated CDK4/CDK6/cyclin D1 complex phosphorylates retinoblastoma (RB) to inhibit its E2F suppression, which governs transcription of genes necessary for S phase. E2F also induces cyclin E expression, then CDK2/cyclin E complex hyperphosphorylates RB to release complete E2F transcriptional activity [26]. By western blotting, we found that the expression of p-RB and CDK4 was notable reduced in TGF-β treated HK-2 cells. Autophagy inhibition caused no further suppression on these proteins (Figure 4). Meanwhile, p-CDK2 and cyclin A and cyclin E have not been modulated by TGF-β stimulation, even when combined with CQ or Baf (Figure 4F). Intriguingly, the TGF-β stimulation increased cyclin D1 and CDK6 level in HK-2 cells, and

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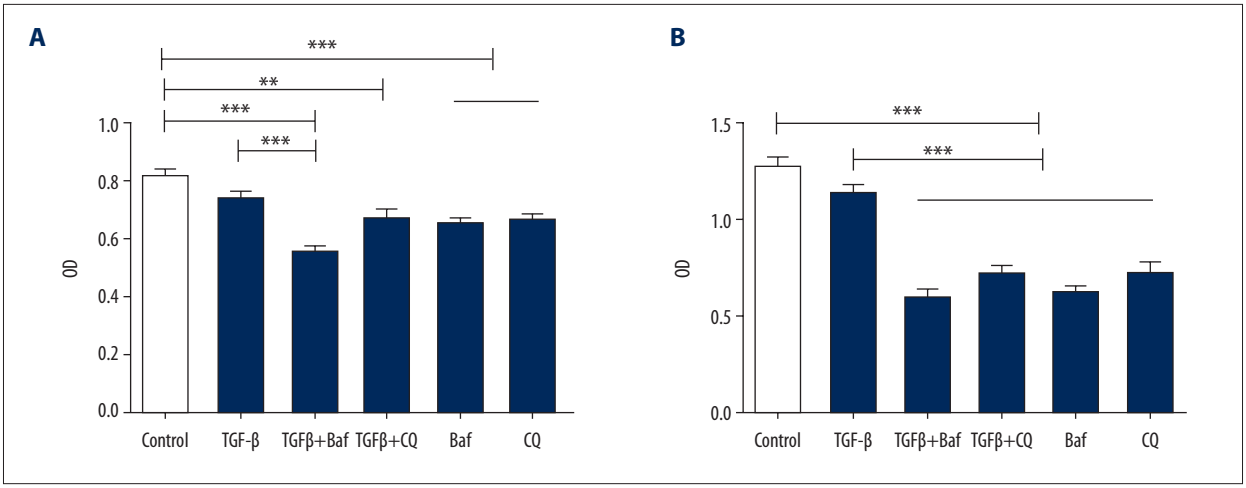

Figure 2. HK-2 cell proliferation as assessed using the CCK-8 method. (**A**) HK-2 cell proliferation after a 24-hour stimulation. (**B**) HK-2 cell proliferation after a 48-hour stimulation. Data represent the mean±SEM for at least 3 independent experiments. ** *P*<0.01, *** *P*<0.001 between 2 groups. HK-2 – human kidney-2; CCK-8 – Cell Counting Kit-8; SEM – standard error of the mean; TGF – transforming growth factor; Baf – bafilomycin A1; CQ – chloroquine; CON – control.

autophagy inhibition further upregulated the expression cyclin D1 and CDK6. In addition, the elevated level of cell cycle inhibitor p21, but not other inhibitors like p53 and p16, in TGF-β treated HK-2 cells was further upregulated by the addition of CQ and Baf (Figure 5). These data indicated that autophagy inhibition enhances G1 arrest in TGF-β treated HK-2 cells by upregulating p21 expression.

Inhibition of autophagy promoted extracellular matrix production in TGF-β treated HK-2 cells

Finally, the effect of autophagy inhibition on TGF-β-induced extracellular matrix protein expression in HK-2 cells was analyzed by western blotting. Figure 6 showed that compared with untreated cells, TGF-β exposed HK-2 cells expressed a higher level of extracellular matrix proteins such as collagen I and fibronectin. In the presence of autophagy inhibitors, TGF-β-induced expression of fibronectin was notably upregulated. Moreover, treatment with autophagy inhibitors like CQ or Baf also slightly elevated the expression of collagen I and mesenchymal markers like α-SMA in TGF-β treated HK-2 cells.

Discussion

In the present study, we found that autophagy inhibition by CQ or Baf sensitized the TECs to growth arrest and extracellular matrix production induced by TGF-β *in vitro*, mainly via arresting cells in the G1 phase. Our findings demonstrate a potential mechanism by which autophagy dysfunction may contribute to development of tubulointerstitial fibrosis.

It is well established that TGF-β suppresses epithelial cell growth [27]. In the present study, we found that TGF-β inhibited the proliferation of HK-2 cells and arrested them in G1 phase via elevating the p21 level. Previous research has identified that TGF-β stimulates G2/M arrest in renal TECs [18]. In addition, TGF-β has also been reported to induce TECs arrested in G1/S phase in an experimental model of acute kidney injury (AKI) [17]. TGF-β may specify different profiles of cycle arrest in TECs in a concentration-dependent manner [28]. Two interconnected processes have revealed to involve in TGF-β causes G1 phase arrest at the R-point, including the repression of expression of certain growth-promoting transcription factors and the induction of expression of specific CDK inhibitors p21 and p15 in epithelial cells [27]. Mechanically, p21 is one of the key downstream mediators involved in TGF-β-induced TECs cell cycle arrest [19,29]. TGF-β stimulation upregulates mRNA and protein level of p21 [30], while deletion of p21 abrogates TGF-β driven growth arrest in TECs [31]. Injured TECs undergoing a transient cycle arrest obtain an opportunity to repair their DNA [32]. For example, the deletion of p21 enhances TECs damage in a mouse model of ischemia-reperfusion-induced AKI [33]. In contrast, a prolonged cell cycle arrest modulates the TECs morphology and increases the aberrant production of pro-fibrotic cytokines [34,35]. During G1/S arrest, the epithelial-to-mesenchymal transition (EMT)-associated phenotype occurs that modestly reduced in total protein synthesis levels, compared to proliferating cells, while increased in the specific mesenchymal markers related to the cellular capacity to migrate and invade distal tissues [36,37]. Other reports also validated that the occurrence of tubulointerstitial fibrosis in diabetic nephropathy requires the induction of specific cell cycle proteins such as p21 that cause G1 phase arrest induced by TGF-β [38]. Thereby, cell cycle arrest in TECs induced

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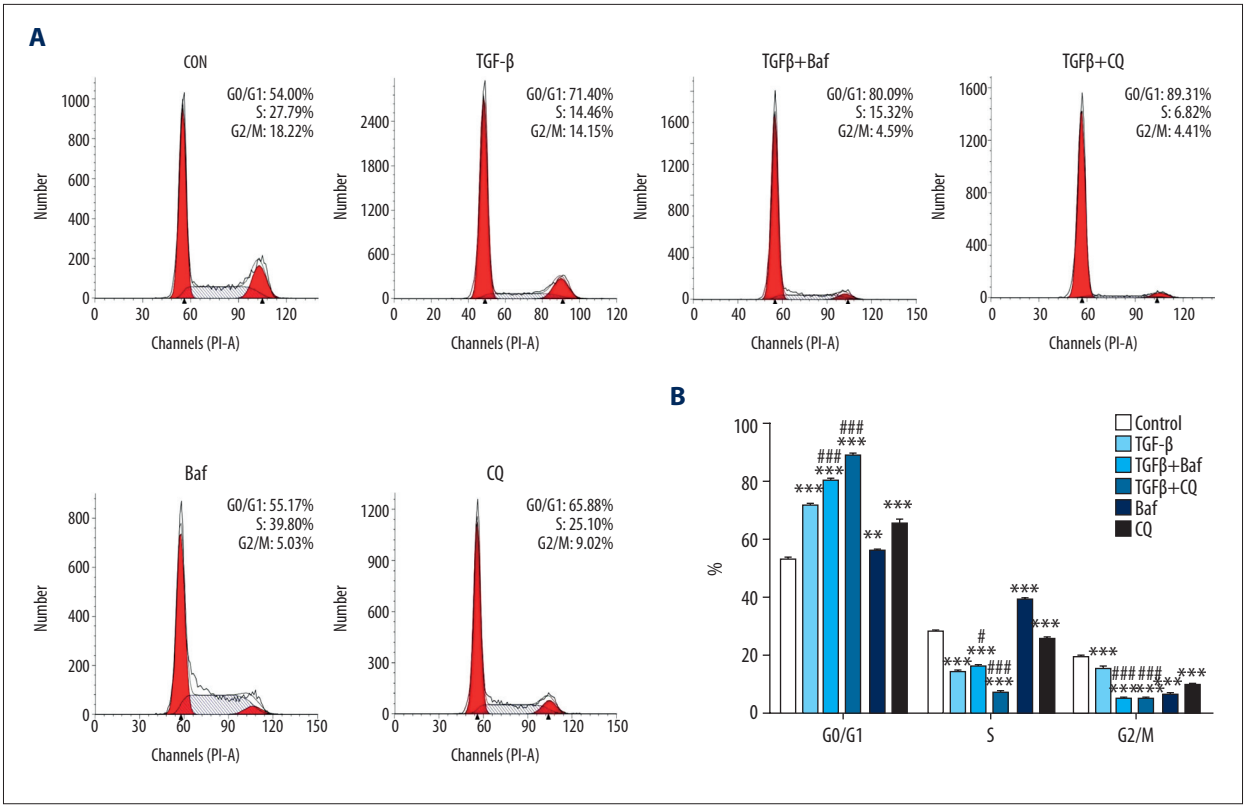
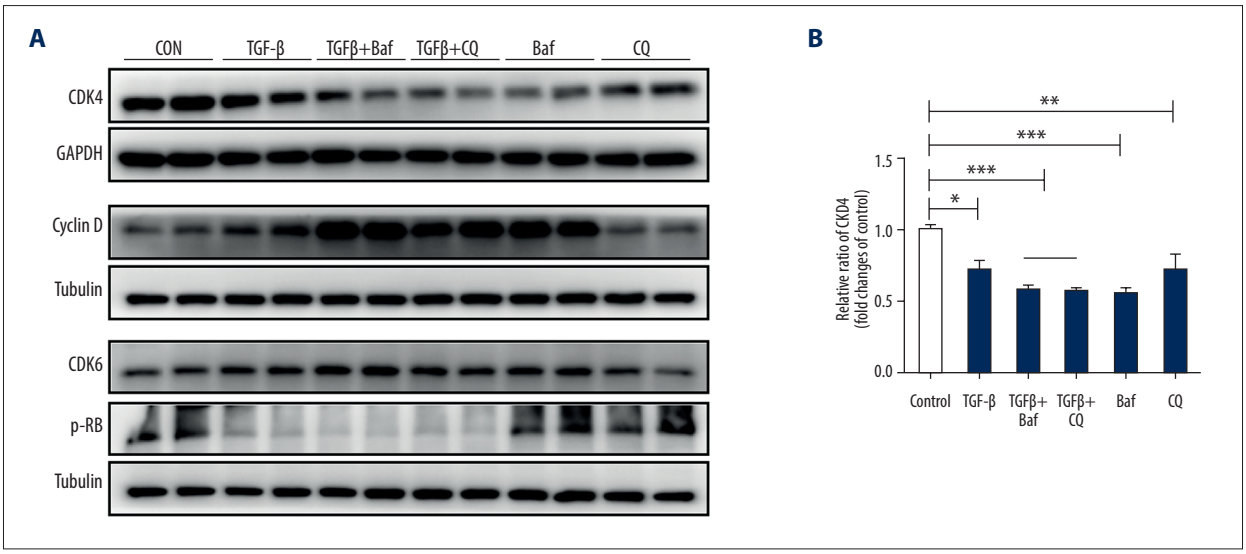


Figure 3. Cell cycle analysis as evaluated by the PI staining and flow cytometry. (**A**) Representative figures of cell cycle analysis by flow cytometry. (**B**) Quantitation of cell cycle distribution. Data represent the mean±SEM for at least 3 independent experiments. ** *P*<0.01, *** *P*<0.001 versus control HK-2 cells; # *P*<0.01, ### *P*<0.001 versus TGF-β treated HK-2 cells. PI – propidium iodide; HK-2 – human kidney-2; SEM – standard error of the mean; TGF – transforming growth factor; Baf – bafilomycin A1; CQ – chloroquine; CON – control.



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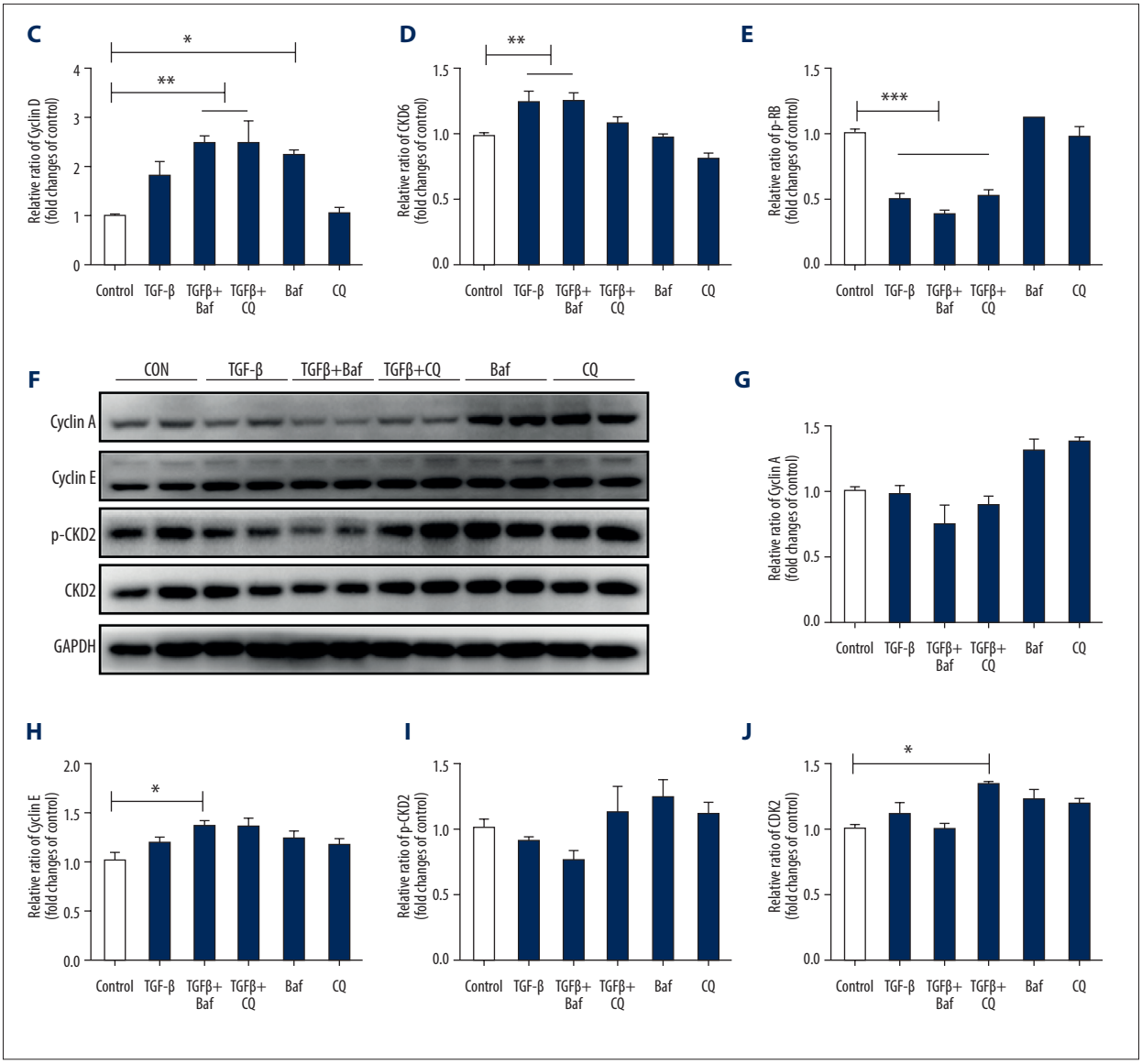

Figure 4. Expression of cell cycle regulatory proteins as detected by western blotting. (**A**) Representative blots of CDK4, CDK6, cyclin D and phosphorylated RB expression levels in HK-2 cells. (**B**) Quantitation of CDK4 expression. (**C**) Quantitation of cyclin D expression. (**D**) Quantitation of CDK6 expression. (**E**) Quantitation of phosphorylated RB expression. (**F**) Representative blots of CDK2, phosphorylated CDK2, cyclin A and cyclin E expression levels in HK-2 cells. (**G**) Quantitation of cyclin A expression. (**H**) Quantitation of cyclin E expression. (**I**) Quantitation of phosphorylated CDK2 expression. (**J**) Quantitation of CDK2 expression. Data represent the mean±SEM for at least 3 independent experiments. * $P<0.05$, ** $P<0.01$, *** $P< 0.001$ between 2 groups. RB – retinoblastoma; HK-2 – human kidney-2; SEM – standard error of the mean; TGF – transforming growth factor; Baf – bafilomycin A1; CQ – chloroquine; CON – control.

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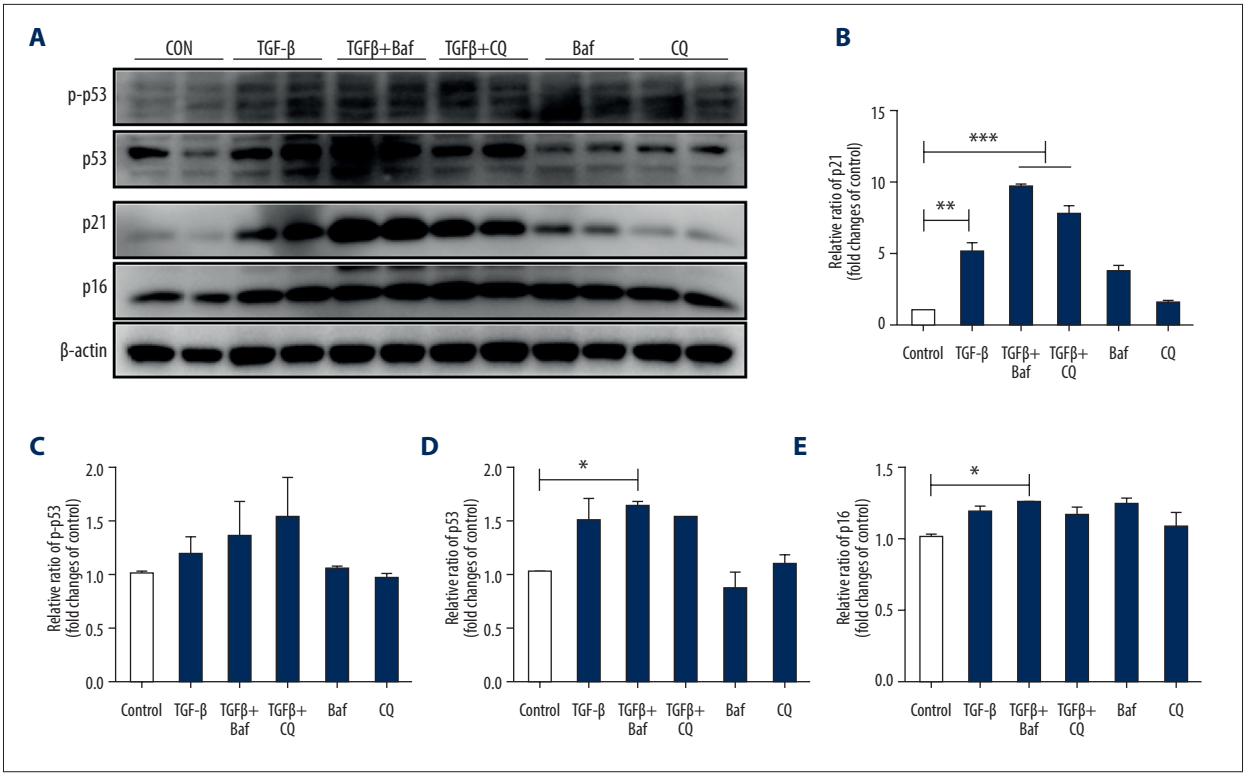

Figure 5. Expression of cell cycle inhibitors as detected by western blotting. (**A**) Representative blots of p53, phosphorylated p53, p16, and p21 expression levels in HK-2 cells. (**B**) Quantitation of p21 expression. (**C**) Quantitation of phosphorylated p53 expression. (**D**) Quantitation of p53 expression. (**E**) Quantitation of p16 expression. Data represent the mean±SEM for at least 3 independent experiments. * $P<0.05$, ** $P<0.01$, *** $P<0.001$ between 2 groups. HK-2 – human kidney-2; SEM – standard error of the mean; TGF – transforming growth factor; Baf – bafilomycin A1; CQ – chloroquine; CON – control.

by TGF-β contributes to the induction of EMT-associated phenotype and tubulointerstitial fibrosis.

The relationship between autophagy regulation and cell cycle progression is still largely unknown. Autophagy seems regulate cell cycle in a cell type-dependent manner [39–41]. Generally, autophagy is dynamically modulated during the progress of cell division [42]. As in the mitosis phase, a cell undergoes rearrangement and divides organelles such as mitochondria to daughter cells, and autophagy is inhibited to avoid the degradation of organelles and chromosomes [43,44]. In response to stress, such as metabolic stress, the cell enters a cell cycle arrest and activates autophagy as a protective mechanism [45]. However, the association of autophagy and cell cycle regulation has not been well understood in the development of tubulointerstitial fibrosis. A previous study has reported that the deletion of ATG5 protein suppresses autophagy and enhances G2/M arrest in TECs, thereby promoting tubulointerstitial fibrosis in obstructive nephropathy [46]. On the contrary, other research showed that autophagy protected against apoptosis but promoted TECs to enter cell cycle arrest and acquire a senescent morphology [47]. These senescent TECs then lead to renal maladaptive repair and contribute to fibrosis [48].

In addition, autophagy-related proteins may regulate cell cycle independent on autophagy induction; for example, ATG7 protein may work with p53 to induce p21 expression [49]. Although genetic technology is a well-accepted method to investigate autophagy, it is rendered incapable when the target protein also served autophagy-independent functions [49]. Thus, in the present study, we used chemical lysosomal inhibitors to suppress the autophagy activity. Different modulation methods and stimulation models may result in these inconsistent conclusions. In the present study, we found that autophagy inhibition by CQ or Baf promoted the TGF-β-induced proliferation suppression and G1 arrest, accompanied with further increased expression of p21 (Figure 7). There were still some unexpected results in our study. First, in TGF-β-treated HK-2 cells, the expression of cyclin D1 was further increased by Baf or CQ, rather than being decreased. The accumulation of cyclin D1 may result from autophagy inhibition [50]. Although cyclin D1 works with CDK4/CDK6 to promote G1/S transition, overexpression of cyclin D1 has been found preventing the cell entry into S phase via binding proliferating cell nuclear antigen (PCNA) and CDK2 [51,52]. Second, the expression of CDK4 was reduced but CDK6 level was consistent during G1 arrest. So TGF-β-induced G1 arrest may be resulted from

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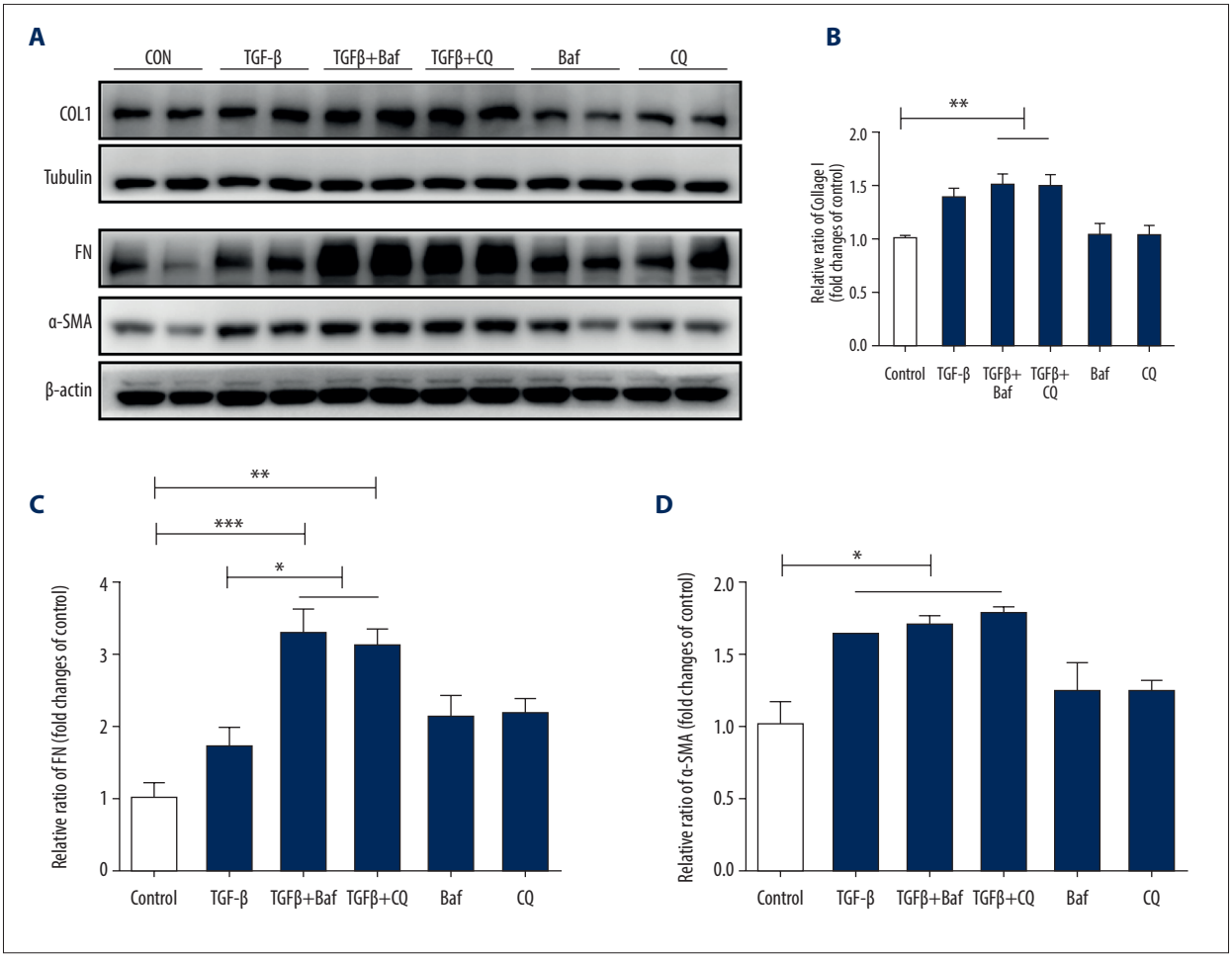

Figure 6. Expression of the extracellular matrix proteins as detected by western blotting. (**A**) Representative blots of collagen I, fibronectin and α-SMA expression levels in HK-2 cells. (**B**) Quantitation of collagen I. (**C**) Quantitation of fibronectin expression. (**D**) Quantitation of α-SMA. Data represent the mean±SEM for at least 3 independent experiments. * $P<0.05$, ** $P<0.01$, *** $P<0.001$ between 2 groups. HK-2 – human kidney-2; SEM – standard error of the mean; TGF – transforming growth factor; Baf – bafilomycin A1; CQ – chloroquine; CON – control.

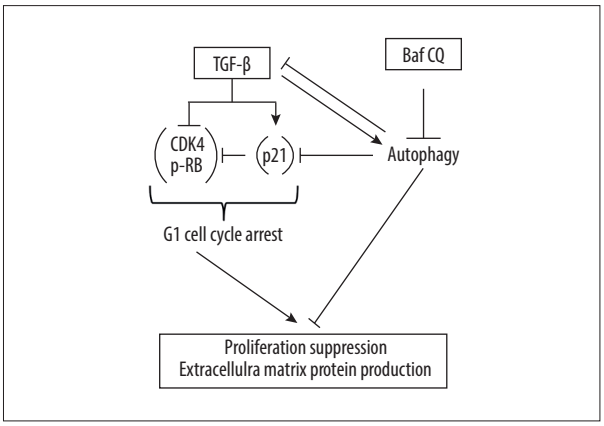

Figure 7. Schematic representation of autophagy inhibition sensitizes TECs to G1 arrest and proliferation suppression induced by TGF-β that promoted extracellular matrix production. TGF-β reduces the expression of CDK4 and p-RB, while increases the p21 expression and all of the changes are further aggravated by autophagy inhibition after exposure to Baf and CQ, resulting in the G1 arrest and proliferation suppression, finally led to the production of extracellular matrix protein in TECs. TECs – tubular epithelial cells; TGF – transforming growth factor; RB – retinoblastoma; Baf – bafilomycin A1; CQ – chloroquine; CON – control.

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the downregulated CDK4 and not CDK6. A previous study has found that p21 inhibits CDK4 activity rather than that of CDK6, despite binding to CDK6 [53].

In the current study, we have identified that autophagy inhibition promoted TGF-β-induced G1 arrest in TECs via enhancing the p21 expression, but the molecular mechanism is partially unclear. On the one hand, autophagy inhibition suppresses TGF-β degradation [24] and thereby promotes TGF-β-mediated p21 induction. On the other hand, CQ or Baf may directly suppress p53 [54] or p21 [55] degradation mediated by the autophagy pathway. The intracellular accumulation of p53 or p21 subsequently causes the cell cycle arrest [56]. The detailed mechanism warrants further investigations in the future.

Conclusions

Taken together, autophagy inhibition sensitizes TECs to G1 arrest and proliferation suppression induced by TGF-β that contributes to the induction of tubulointerstitial fibrosis, this is one of the mechanisms by which autophagy may be involved in the development of tubulointerstitial fibrosis.

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Cconflicts of interests

None.

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